Prime 1.5

Quick Start Guide

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Document Conventions

In addition to the use of italics for names of documents, the font conventions that are used in this document are summarized in the table below.

Table 1.1.

Font	Example	Use
Sans serif	Project Table	Names of GUI features, such as panels, menus, menu items, buttons, and labels
Monospace	`$SCHRODINGER/maestro`	File names, directory names, commands, environment variables, and screen output
Italic	*filename*	Text that the user must replace with a value
Sans serif uppercase	CTRL+H	Keyboard keys

In descriptions of command syntax, the following UNIX conventions are used: braces `{ }` enclose a choice of required items, square brackets `[ ]` enclose optional items, and the bar symbol | separates items in a list from which one item must be chosen. Lines of command syntax that wrap should be interpreted as a single command.

In this document, to *type* text means to type the required text in the specified location, and to *enter* text means to type the required text, then press the ENTER key.

References to literature sources are given in square brackets, like this: [10].

Introduction

This manual provides a tutorial introduction to using the Prime™ protein structure prediction suite. It includes a general introduction to Maestro™ followed by tutorial exercises for the Prime–Structure Prediction Comparative Modeling Path, the Prime–Structure Prediction Threading Path. For a tutorial introduction to the Induced Fit Docking protocol, which uses Prime and Glide™, see the document *Induced Fit Docking*.

Maestro is the graphical interface for Schrödinger products. The two Prime modules, Prime–Structure Prediction and Prime–Refinement, are run from Maestro panels which are opened from the Maestro Applications menu. Prime also uses the main Maestro window to display 3D structures and the Maestro Project Facility to handle information about the structures it produces. If you are not familiar with Maestro, or would like an overview that includes features that are new in this beta release, see Chapter 2. For more information on using Maestro, see the Maestro online help or the *Maestro User Manual*.

The Prime modules are briefly described in the tutorial chapters. For more information about Prime features, see the *Prime User Manual*.

It is assumed that you have already installed Maestro 7.5, Prime 1.5, and supporting third-party programs and databases (PDB, BLAST, HMMER/Pfam) from the Schrödinger CDs. In addition, it is assumed that you have downloaded and installed the optional (but highly recommended) third-party secondary structure prediction program PSIPRED. To find out how to obtain third-party programs, go to the Third Party Programs page of our website.

Introduction to Maestro

Maestro is the graphical user interface for all of Schrödinger's products: CombiGlide™, Epik™, Glide™, Impact™, Jaguar™, Liaison™, LigPrep™, MacroModel®, Phase™, Prime™, QikProp™, QSite™, and Strike™. It contains tools for building, displaying, and manipulating chemical structures; for organizing, loading, and storing these structures and associated data; and for setting up, monitoring, and visualizing the results of calculations on these structures. This chapter provides a brief introduction to Maestro and some of its capabilities. For more information on any of the topics in this chapter, see the *Maestro User Manual*.

2.1 General Interface Behavior

Most Maestro panels are amodal: more than one panel can be open at a time, and a panel need not be closed for an action to be carried out. Each Maestro panel has a Close button so you can hide the panel from view.

Maestro supports the mouse functions common to many graphical user interfaces. The left button is used for choosing menu items, clicking buttons, and selecting objects by clicking or dragging. This button is also used for resizing and moving panels. The right button displays a shortcut menu. Other common mouse functions are supported, such as using the mouse in combination with the SHIFT or CTRL keys to select a range of items and select or deselect a single item without affecting other items.

In addition, the mouse buttons are used for special functions described later in this chapter. These functions assume that you have a three-button mouse. If you have a two-button mouse, ensure that it is configured for three-button mouse simulation (the middle mouse button is simulated by pressing or holding down both buttons simultaneously).

2.2 Starting Maestro

Before starting Maestro, you must first set the `SCHRODINGER` environment variable to point to the installation directory. To set this variable, enter the following command at a shell prompt:

csh/tcsh: `setenv SCHRODINGER` *installation-directory*

bash/ksh: `export SCHRODINGER=`*installation-directory*

You might also need to set the DISPLAY environment variable, if it is not set automatically when you log in. To determine if you need to set this variable, enter the command:

```
echo $DISPLAY
```

If the response is a blank line, set the variable by entering the following command:

csh/tcsh: `setenv DISPLAY` *display-machine-name*`:0.0`

bash/ksh: `export DISPLAY=`*display-machine-name*`:0.0`

After you set the SCHRODINGER and DISPLAY environment variables, you can start Maestro using the command:

```
$SCHRODINGER/maestro options
```

If you add the $SCHRODINGER directory to your path, you only need to enter the command maestro. Options for this command are given in Section 2.1 of the *Maestro User Manual*.

The directory from which you started Maestro is Maestro's current working directory, and all data files are written to and read from this directory unless otherwise specified (see Section 2.8 on page 25). You can change directories by entering the following command in the command input area (see page 6) of the main window:

```
cd directory-name
```

where *directory-name* is either a full path or a relative path.

2.3 The Maestro Main Window

The Maestro main window is shown in Figure 2.1 on page 5. The main window components are listed below.

The following components are always visible:

- **Title bar**—displays the Maestro version, the project name (if there is one) and the current working directory.
- **Auto-Help**—automatically displays context-sensitive help.
- **Menu bar**—provides access to panels.
- **Workspace**—displays molecular structures and other 3D graphical objects.

The following components can be displayed or hidden by choosing the component from the Display menu. Your choice of which main window components are displayed is persistent between Maestro sessions.

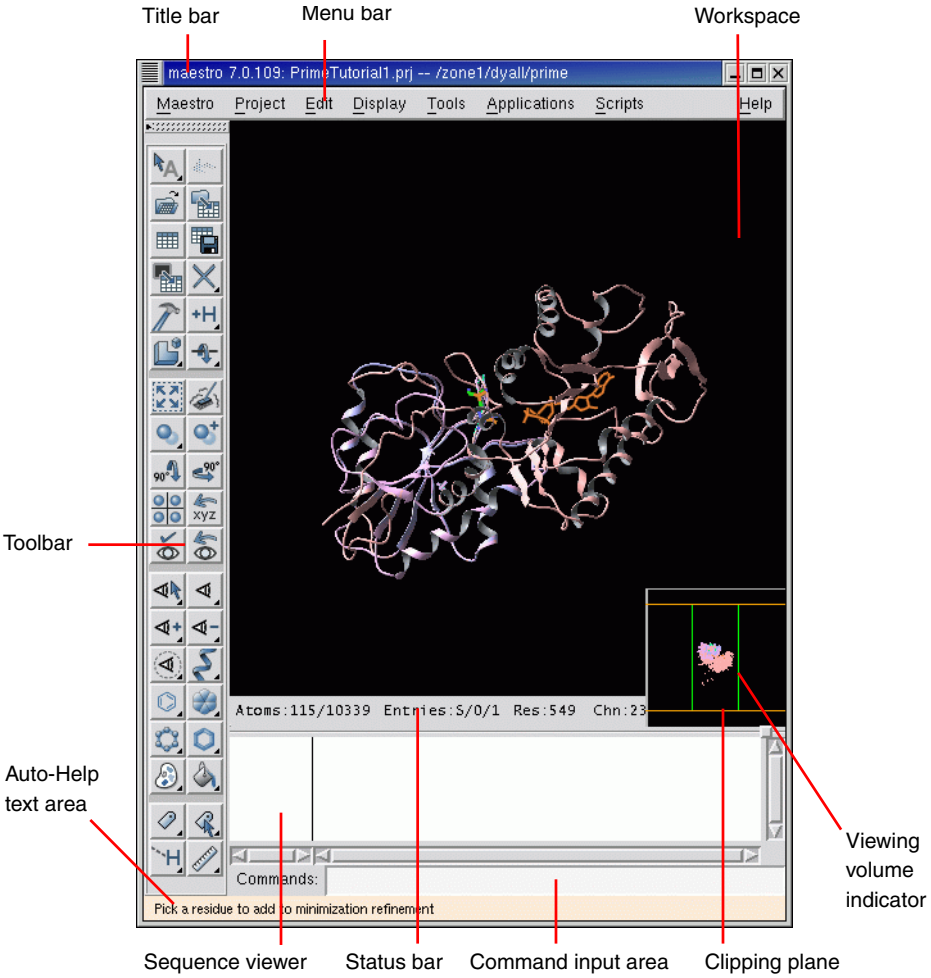

Figure 2.1. The Maestro main window.

- **Toolbar**—contains buttons for many common tasks and provides tools for displaying and manipulating structures, as well as organizing the Workspace.
- **Status bar**—displays information about a particular atom, or about structures in the Workspace, depending on where the pointer pauses (see Section 2.5 of the *Maestro User Manual* for details):
  - **Atom**—displays the chain, residue number, element, PDB atom name, formal charge, and title or entry name (this last field is set by choosing Preferences from the Maestro menu and selecting the Feedback folder).

  - **Workspace**—displays the number of atoms, entries, residues, chains, and molecules in the Workspace.

- **Clipping planes window**—displays a small, top view of the Workspace and shows the clipping planes and viewing volume indicators.
- **Sequence viewer**—shows the sequences for proteins displayed in the Workspace. See Section 2.6 of the *Maestro User Manual* for details.
- **Command input area**—provides a place to enter Maestro commands.

When a distinction between components in the main window and those in other panels is needed, the term *main* is applied to the main window components (e.g., main toolbar).

You can expand the Workspace to occupy the full screen, by pressing CTRL+=. All other components and panels are hidden. To return to the previous display, press CTRL+= again.

2.3.1 The Menu Bar

The menus on the main menu bar provide access to panels, allow you to execute commands, and control the appearance of the Workspace. The main menus are as follows:

- Maestro—save or print images in the Workspace, execute system commands, save or load a panel layout, set preferences, set up Maestro command aliases, and quit Maestro.
- Project—open and close projects, import and export structures, make a snapshot, and annotate a project. These actions can also be performed from the Project Table panel. For more information, see Section 2.4 on page 11.
- Edit—undo actions, build and modify structures, define command scripts and macros, and find atoms in the Workspace.
- Display—control the display of the contents of the Workspace, arrange panels, and display or hide main window components.
- Tools—group atoms; measure, align, and superimpose structures; and view and visualize data.
- Applications—set up, submit, and monitor jobs for Schrödinger's computational programs. Some products have a submenu from which you can choose the task to be performed.
- Scripts—manage and install Python scripts that come with the distribution and scripts that you create yourself. (See Chapter 13 of the *Maestro User Manual* for details.)
- Help—open the Help panel, the PDF documentation index, or information panels; run a demonstration; and display or hide Balloon Help (tooltips).

2.3.2 The Toolbar

The main toolbar contains three kinds of buttons for performing common tasks:

Action—Perform a simple task, like clearing the Workspace.

Display—Open or close a panel or open a dialog box, such as the Project Table panel.

Menu—Display a *button menu*. These buttons have a triangle in the lower right corner.

There are four types of items on button menus, and all four types can be on the same menu (see Figure 2.2):

- **Action**—Perform an action immediately.
- **Display**—Open a panel or dialog box.
- **Object types for selection**—Choose Atoms, Bonds, Residues, Chains, Molecules, or Entries, then click on an atom in the Workspace to perform the action on all the atoms in that structural unit.

  The object type is marked on the menu with a red diamond and the button is indented to indicate the action to be performed.
- **Other setting**—Set a state, choose an attribute, or choose a parameter and click on atoms in the Workspace to display or change that parameter.

The toolbar buttons are described below. Some descriptions refer to features not described in this chapter. See the *Maestro User Manual* for a fuller description of these features.

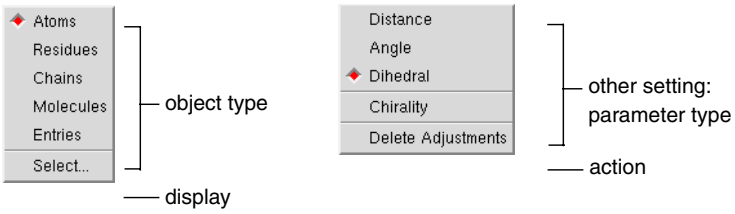

Figure 2.2. The Workspace selection ***button menu and the*** Adjust distances, angles or dihedrals ***button menu.***

Workspace selection
– Choose an object type for selecting
– Open the Atom Selection dialog box

Undo/Redo
Undo or redo the last action. Performs the same function as the Undo item on the Edit menu, and changes to an arrow pointing in the opposite direction when an Undo has been performed, indicating that its next action is Redo.

Open a project
Open the Open Project dialog box.

Import structures
Open the Import panel.

Open/Close Project Table
Open the Project Table panel or close it if it is open.

Save as
Open the Save Project As dialog box, to save the project with a new name.

Create entry from Workspace
Open a dialog box in which you can create an entry in the current project using the contents of the Workspace.

Delete
– Choose an object type for deletion
– Delete hydrogens and waters
– Open the Atom Selection dialog box
– Delete other items associated with the structures in the Workspace
– Click to select atoms to delete
– Double-click to delete all atoms

Open/Close Build panel
Open the Build panel or close it if it is open.

Add hydrogens
– Choose an object type for applying a hydrogen treatment
– Open the Atom Selection dialog box
– Click to select atoms to treat
– Double-click to apply to all atoms

Local transformation
– Choose an object type for transforming
– Click to select atoms to transform
– Open the Advanced Transformations panel

Adjust distances, angles or dihedrals
– Choose a parameter for adjusting
– Delete adjustments

Fit to screen
Scale the displayed structure to fit into the Workspace and reset the center of rotation.

Clear Workspace
Clear all atoms from the Workspace.

Set fog display state
Choose a fog state. Automatic means fog is on when there are more than 40 atoms in the Workspace, otherwise it is off.

Enhance depth cues
Optimize fogging and other depth cues based on what is in the Workspace.

Rotate around X axis by 90 degrees
Rotate the Workspace contents around the X axis by 90 degrees.

Rotate around Y axis by 90 degrees
Rotate the Workspace contents around the Y axis by 90 degrees.

Tile entries
Arrange entries in a rectangular grid in the Workspace.

Reset Workspace
Reset the rotation, translation, and zoom of the Workspace to the default state.

Save view
Save the current view of the Workspace: orientation, location, and zoom.

Restore view
Restore the last saved view of the Workspace: orientation, location, and zoom.

Display only selected atoms
– Choose an object type for displaying
– Click to select atoms to display
– Double-click to display all atoms

Display only
– Choose a predefined atom category
– Open the Atom Selection dialog box

Also display
– Choose a predefined atom category
– Open the Atom Selection dialog box

Undisplay
– Choose a predefined atom category
– Open the Atom Selection dialog box

Display residues within N angstroms of currently displayed atoms
– Choose a radius
– Open a dialog box to set a value

Show, hide, or color ribbons
– Choose to show or hide ribbons
– Choose a color scheme for coloring ribbons

Draw bonds in wire
– Choose an object type for drawing bonds in wire representation
– Open the Atom Selection dialog box
– Click to select atoms for representation
– Double-click to apply to all atoms

Draw atoms in CPK
– Choose an object type for drawing bonds in CPK representation
– Open the Atom Selection dialog box
– Click to select atoms for representation
– Double-click to apply to all atoms

Draw atoms in Ball & Stick
– Choose an object type for drawing bonds in Ball & Stick representation
– Open the Atom Selection dialog box
– Click to select atoms for representation
– Double-click to apply to all atoms

Draw bonds in tube
– Choose an object type for drawing bonds in tube representation
– Open the Atom Selection dialog box
– Click to select atoms for representation
– Double-click to apply to all atoms

Color all atoms by scheme
Choose a predefined color scheme.

Color residue by constant color
– Choose a color for applying to residues
– Click to select residues to color
– Double-click to color all atoms

Label atoms
– Choose a predefined label type
– Delete labels

Label picked atoms
– Choose an object type for labeling atoms
– Open the Atom Selection dialog box
– Open the Atom Labels panel at the Composition folder
– Delete labels
– Click to select atoms to label
– Double-click to label all atoms

Display H-bonds
– Choose bond type:
intra—displays H-bonds within the selected molecule
inter—displays H-bonds between the selected molecule and all other atoms.
– Delete H-bonds
– Click to select molecule

Measure distances, angles or dihedrals
– Choose a parameter for displaying measurements
– Delete measurements
– Click to select atoms for measurement

2.3.3 Mouse Functions in the Workspace

The left mouse button is used for selecting objects. You can either click on a single atom or bond, or you can drag to select multiple objects. The right mouse button opens shortcut menus, which are described in Section 2.7 of the *Maestro User Manual*.

The middle and right mouse buttons can be used on their own and in combination with the SHIFT and CTRL keys to perform common operations, such as rotating, translating, centering, adjusting, and zooming.

Table 2.1. Mapping of Workspace operations to mouse actions.

Mouse Button	Keyboard	Motion	Action
Left		click, drag	Select
Left	SHIFT	click, drag	Toggle the selection
Middle		drag	Rotate about X and Y axes Adjust bond, angle, or dihedral
Middle	SHIFT	drag vertically	Rotate about X axis
Middle	SHIFT	drag horizontally	Rotate about Y axis
Middle	CTRL	drag horizontally	Rotate about Z axis
Middle	SHIFT + CTRL	drag horizontally	Zoom
Right		click	Spot-center on selection
Right		click and hold	Display shortcut menu
Right		drag	Translate in the X-Y plane
Right	SHIFT	drag vertically	Translate along the X axis
Right	SHIFT	drag horizontally	Translate along the Y axis
Right	CTRL	drag horizontally	Translate along the Z axis
Middle & Right		drag horizontally	Zoom

2.3.4 Shortcut Key Combinations

Some frequently used operations have been assigned shortcut key combinations. The shortcuts available in the main window are described in Table 2.2.

Table 2.2. Shortcut keys in the Maestro main window.

Keys	Action	Equivalent Menu Choices
CTRL+B	Open Build panel	Edit > Build
CTRL+C	Create entry	Project > Create Entry From Workspace
CTRL+E	Open Command Script Editor panel	Edit > Command Script Editor
CTRL+F	Open Find Atoms panel	Edit > Find
CTRL+H	Open Help panel	Help > Help
CTRL+I	Open Import panel	Project > Import Structures
CTRL+M	Open Measurements panel	Tools > Measurements
CTRL+N	Create new project	Project > New
CTRL+O	Open project	Project > Open
CTRL+P	Print	Maestro > Print
CTRL+Q	Quit	Maestro > Quit
CTRL+S	Open Sets panel	Tools > Sets
CTRL+T	Open Project Table panel	Project > Show Table
CTRL+W	Close project	Project > Close
CTRL+Z	Undo/Redo last command	Edit > Undo/Redo
CTRL+=	Enter and exit full screen mode (Workspace occupies full screen)	None

2.4 Maestro Projects

All the work you do in Maestro is done within a *project*. A project consists of a set of *entries*, each of which contains one or more chemical structures and their associated data. In any Maestro session, there can be only one Maestro project open. If you do not specify a project when you start Maestro, a *scratch* project is created. You can work in a scratch project without saving it, but you must save it in order to use it in future sessions. When you save or close a project, all the view transformations (rotation, translation, and zoom) are saved with it. When you close a project, a new scratch project is automatically created.

Likewise, if there is no entry displayed in the Workspace, Maestro creates a *scratch* entry. Structures that you build in the Workspace constitute a scratch entry until you save the structures as project entries. The scratch entry is not saved with the project unless you explicitly add it to the project. However, you can use a scratch entry as input for some calculations.

To add a scratch entry to a project, do one of the following:

- Click the Create entry from Workspace button:

- Choose Create Entry from Workspace from the Project menu.
- Press CTRL+C.

In the dialog box, enter a name and a title for the entry. The entry name is used internally to identify the entry and can be modified by Maestro. The title can be set or changed by the user, but is not otherwise modified by Maestro.

Once an entry has been incorporated into the project, its structures and their data are represented by a row in the Project Table. Each row contains the row number, an icon indicating whether the entry is displayed in the Workspace (the In column), the entry title, a button to open the Surfaces panel if the entry has surfaces, the entry name, and any entry properties. The row number is not a property of the entry.

Entries can be collected into groups, and the members of the group can be displayed or hidden. Most additions of multiple entries to the Project Table are done as entry groups.

You can use entries as input for all of the computational programs—Glide, Impact, Jaguar, Liaison, LigPrep, MacroModel, Phase, Prime, QikProp, QSite, and Strike. You can select entries as input for the ePlayer, which displays the selected structures in sequence. You can also duplicate, combine, rename, and sort entries; create properties; import structures as entries; and export structures and properties from entries in various formats.

To open the Project Table panel, do one of the following:

- Click the Open/Close Project Table button on the toolbar

- Choose Show Table from the Project menu
- Press CTRL+T.

The Project Table panel contains a menu bar, a toolbar, and the table itself.

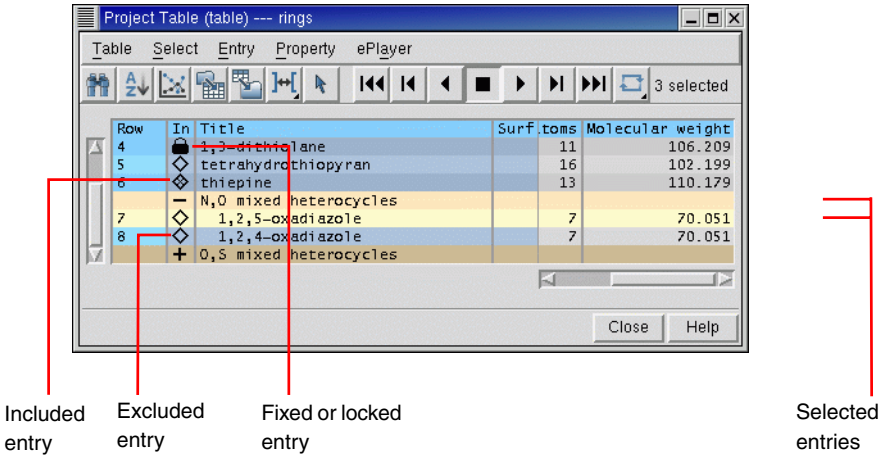

Figure 2.3. The** Project Table **panel.

2.4.1 The Project Table Toolbar

The Project Table toolbar contains two groups of buttons and a status display. The first set of buttons opens various panels that allow you to perform functions on the entries in the Project Table. The second set of buttons controls the ePlayer, which "plays through" the selected structures: each structure is displayed in the Workspace in sequence, at a given time interval. See Section 2.3.2 on page 7 for a description of the types of toolbar buttons. The buttons are described below.

Find
Open the Find panel for locating alphanumeric text in any column of the Project Table, except for the row number.

Sort
Open the Sort panel for sorting entries by up to three properties.

Plot
Open the Plot panel for plotting entry properties.

Import Structure
Open the Import panel for importing structures into the project.

Export Structure
Open the Export panel for exporting structures to a file.

Columns
Choose an option for adjusting the column widths.

Select only
Open the Entry Selection dialog box for selecting entries based on criteria for entry properties.

Go to start
Display the first selected structure.

Previous
Display the previous structure in the list of selected structures.

Play backward
Display the selected structures in sequence, moving toward the first.

Stop
Stop the ePlayer.

Play forward
Display the selected structures in sequence, moving toward the last.

Next
Display the next structure in the list of selected structures.

Go to end
Display the last selected structure.

Loop
Choose an option for repeating the display of the structures. Single Direction displays structures in a single direction, then repeats. Oscillate reverses direction each time the beginning or end of the list is reached.

The status display, to the right of the toolbar buttons, shows the number of selected entries. When you pause the cursor over the status display, the Balloon Help shows the total number of entries, the number shown in the table, the number selected, and the number included in the Workspace.

2.4.2 The Project Table Menus

- Table—find text, sort entries, plot properties, import and export structures, and configure the Project Table.
- Select—select all entries, none, invert your selection, or select classes of entries using the Entry Selection dialog box and the Filter panel.

- Entry—include or exclude entries from the Workspace, display or hide entries in the Project Table, and perform various operations on the selected entries.
- Property—display and manipulate entry properties in the Project Table.
- ePlayer—view entries in succession, stop, reverse, and set the ePlayer options.

2.4.3 Selecting Entries

Many operations in Maestro are performed on the entries selected in the Project Table. The Project Table functions much like any other table: select rows by clicking, shift-clicking, and control-clicking. However, because clicking in an editable cell of a selected row enters edit mode, you should click in the Row column to select entries. See Section 2.4.5 on page 16 for more information on mouse actions in the Project Table. There are shortcuts for selecting classes of entries on the Select menu.

In addition to selecting entries manually, you can select entries that meet a combination of conditions on their properties. Such combinations of conditions are called *filters*. Filters are Entry Selection Language (ESL) expressions and are evaluated at the time they are applied. For example, if you want to set up a Glide job that uses ligands with a low molecular weight (say, less than 300) and that has certain QikProp properties, you can set up a filter and use it to select entries for the job. If you save the filter, you can use it again on a different set of ligands that meet the same selection criteria.

To create a filter:

1. Do one of the following:
   - Choose Only, Add, or Deselect from the Select menu.
   - Click the Entry selection button on the toolbar.

   
2. In the Properties folder, select a property from the property list, then select a condition.
3. Combine this selection with the current filter by clicking Add, Subtract, or Intersect. These buttons perform the Boolean operations OR, AND NOT, and AND on the corresponding ESL expressions.
4. To save the filter for future use click Create Filter, enter a name, and click OK.
5. Click OK to apply the filter immediately.

2.4.4 Including Entries in the Workspace

In addition to selecting entries, you can also use the Project Table to control which entries are displayed in the Workspace. An entry that is displayed in the Workspace is *included* in the Workspace; likewise, an entry that is not displayed is *excluded*. Included entries are marked by an X in the diamond in the In column; excluded entries are marked by an empty diamond. Entry inclusion is completely independent of entry selection.

To include or exclude entries, click, shift-click, or control-click in the In column of the entries, or select entries and choose Include or Exclude from the Entry menu. Inclusion with the mouse works just like selection: when you include an entry by clicking, all other entries are excluded.

It is sometimes useful to keep one entry in the Workspace and include others one by one: for example, a receptor and a set of ligands. You can fix the receptor in the Workspace by selecting it in the Project Table and choosing Fix from the Entry menu or by pressing CTRL+F. A padlock icon replaces the diamond in the In column to denote a *fixed* entry. To remove a fixed entry from the Workspace, you must exclude it explicitly (CTRL+X). It is not affected by the inclusion or exclusion of other entries. Fixing an entry affects only its inclusion; you can still rotate, translate, or modify the structure.

2.4.5 Mouse Functions in the Project Table

The Project Table supports the standard use of shift-click and control-click to select objects. This behavior applies to the selection of entries and the inclusion of entries in the Workspace. You can also drag to resize rows and columns and to move rows.

You can drag a set of non-contiguous entries to reposition them in the Project Table. When you release the mouse button, the entries are placed after the first unselected entry that precedes the entry on which the cursor is resting. For example, if you select entries 2, 4, and 6, and release the mouse button on entry 3, these three entries are placed after entry 1, because entry 1 is the first unselected entry that precedes entry 3. To move entries to the top of the table, drag them above the top of the table; to move entries to the end of the table, drag them below the end of the table.

A summary of mouse functions in the Project Table is provided in Table 2.3.

Table 2.3. Mouse operations in the Project Table.

Task	Mouse Operation
Change a Boolean property value	Click repeatedly in a cell to cycle through the possible values (On, Off, Clear)
Display the Entry menu for an entry	Right-click anywhere in the entry. If the entry is not selected, it becomes the selected entry. If the entry is selected, the action is applied to all selected entries.
Display a version of the Property menu for a property	Right-click in the column header
Edit the text or the value in a table cell	Click in the cell and edit the text or value
Include an entry in the Workspace, exclude all others	Click the In column of the entry
Move selected entries	Drag the entries
Paste text into a table cell	Middle-click
Resize rows or columns	Drag the boundary with the middle mouse button
Select an entry, deselect all others	For an unselected entry, click anywhere in the row except the In column; for a selected entry, click the row number.
Select or include multiple entries	Click the first entry then shift-click the last entry
Toggle the selection or inclusion state	Control-click the entry or the In column

2.4.6 Project Table Shortcut Keys

Some frequently used project operations have been assigned shortcut key combinations. The shortcuts, their functions, and their menu equivalents are listed in Table 2.4.

Table 2.4. Shortcut keys in the Project Table.

Keys	Action	Equivalent Menu Choices
CTRL+A	Select all entries	Select > All
CTRL+F	Fix entry in Workspace	Entry > Fix
CTRL+I	Open Import panel	Table > Import Structures
CTRL+N	Include only selected entries	Entry > Include Only
CTRL+U	Deselect all entries	Select > None
CTRL+X	Exclude selected entries	Entry > Exclude
CTRL+Z	Undo/Redo last command	Edit > Undo/Redo in main window

2.5 Building a Structure

After you start Maestro, the first task is usually to create or import a structure. You can open existing Maestro projects or import structures from other sources to obtain a structure, or you can build your own. To open the Build panel, do one of the following:

- Click the Open/Close Build panel button in the toolbar:

- Choose Build from the Edit menu.
- Press CTRL+B.

The Build panel allows you to create structures by drawing or placing atoms or fragments in the Workspace and connecting them into a larger structure, to adjust atom positions and bond orders, and to change atom properties. This panel contains a toolbar and three folders.

2.5.1 Placing and Connecting Fragments

The Build panel provides several tools for creating structures in the Workspace. You can place and connect fragments, or you can draw a structure freehand.

To place a fragment in the Workspace:

1. Select Place.
2. Choose a fragment library from the Fragments menu.
3. Click a fragment.
4. Click in the Workspace where you want the fragment to be placed.

To connect fragments in the Workspace, do one of the following:

- Place another fragment and connect them using the Connect & Fuse panel, which you open from the Edit menu on the main menu bar or with the Display Connect & Fuse panel on the Build toolbar.

- Replace one or more atoms in the existing fragment with another fragment by selecting a fragment and clicking in the Workspace on the main atom to be replaced.
- Grow another fragment by selecting Grow in the Build panel and clicking the fragment you want to add in the Fragments folder.

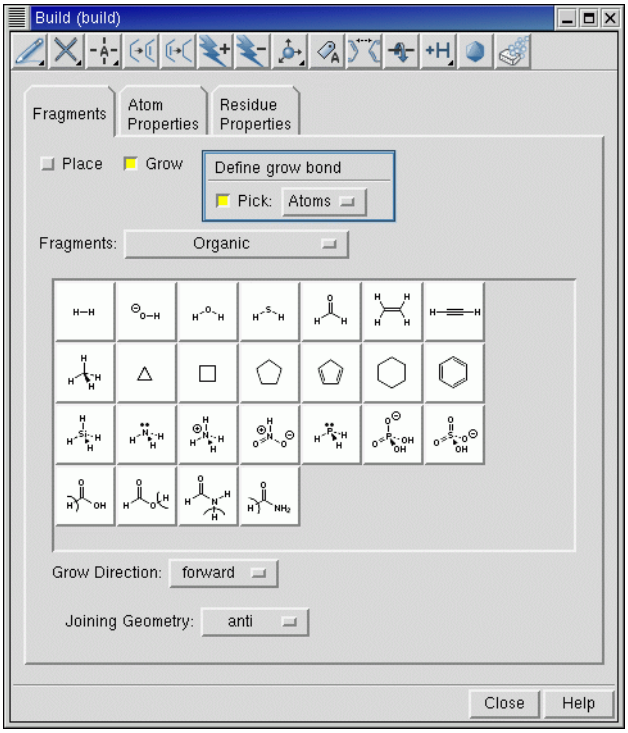

Figure 2.4. The* Build *panel.

Grow mode uses predefined rules to connect a fragment to the *grow bond*. The grow bond is marked by a green arrow. The new fragment replaces the atom at the head of the arrow on the grow bond and all atoms attached to it. To change the grow bond, choose Bonds from the Pick option menu in the Build panel and click on the desired grow bond in the Workspace. The arrow points to the atom nearest to where you clicked.

To draw a structure freehand:

1. Choose an element from the Draw button menu on the Build panel toolbar:

   

2. Click in the Workspace to place an atom of that element.
3. Click again to place another atom and connect it to the previous atom.
4. Continue this process until you have drawn the structure.
5. Click the active atom again to finish drawing.

2.5.2 Adjusting Properties

In the Atom Properties folder, you can change the properties of the atoms in the Workspace. For each item on the Property option menu—Element, Atom Type (MacroModel), Partial Charge, PDB Atom Name, Grow Name, and Atom Name—there is a set of tools you can use to change the atom properties. For example, the Element tools consist of a periodic table from which you can choose an element and select an atom to change it to an atom of the selected element.

Similarly, the Residue Properties folder provides tools for changing the properties of residues: the Residue Number, the Residue Name, and the Chain Name.

To adjust bond lengths, bond angles, dihedral angles, and chiralities during or after building a structure, use the Adjust distances, angles or dihedrals button on the main toolbar:

You can also open the Adjust panel from this button menu, from the Display Adjust panel button on the Build panel toolbar (which has the same appearance as the above button) or from the Edit menu in the main window.

2.5.3 The Build Panel Toolbar

The toolbar of the Build panel provides quick access to tools for drawing and modifying structures and labeling atoms. See Section 2.3.2 on page 7 for a description of the types of toolbar buttons. The toolbar buttons and their use are described below.

Free-hand drawing
Choose an element for drawing structures freehand in the Workspace (default C). Each click in the Workspace places an atom and connects it to the previous atom.

Delete
Choose an object for deleting. Same as the Delete button on the main toolbar, see page 8.

Set element
Choose an element for changing atoms in the Workspace (default C). Click an atom to change it to the selected element.

Increment bond order
Select a bond to increase its bond order by one, to a maximum of 3.

Decrement bond order
Select a bond to decrease its bond order by one, to a minimum of 0.

Increment formal charge
Select an atom to increase its formal charge by one.

Decrement formal charge
Select an atom to decrease its formal charge by one.

Move
Choose a direction for moving atoms, then click the atom to be moved. Moves in the XY plane are made by clicking the new location. Moves in the Z direction are made in 0.5 Å increments.

Label
Apply heteroatom labels as you build a structure. The label consists of the element name and formal charge, and is applied to atoms other than C and H.

Display Connect & Fuse panel
Open the Connect & Fuse panel so you can connect structures (create bonds between structures) or fuse structures (replace atoms of one structure with those of another).

Display Adjust panel
Open the Adjust panel so you can change bond lengths, bond angles, dihedral angles, or atom chiralities.

Add hydrogens
Choose an atom type for applying the current hydrogen treatment. Same as the Add hydrogens button on the main toolbar, see page 8.

Geometry Symmetrizer
Open the Geometry Symmetrizer panel for symmetrizing the geometry of the structure in the Workspace.

Geometry Cleanup
Clean up the geometry of the structure in the Workspace.

2.6 Selecting Atoms

Maestro has a powerful set of tools for selecting atoms in a structure: toolbar buttons, picking tools in panels, and the Atom Selection dialog box. These tools allow you to select atoms in two ways:

- Select atoms first and apply an action to them
- Choose an action first and then select atoms for that action

2.6.1 Toolbar Buttons

The small triangle in the lower right corner of a toolbar button indicates that the button contains a menu. Many of these buttons allow you to choose an object type for selecting: choose Atoms, Bonds, Residues, Chains, Molecules, or Entries, then click on an atom in the Workspace to perform the action on all the atoms in that structural unit.

For example, to select atoms with the Workspace selection toolbar button:

1. Choose Residues from the Workspace selection button menu:

   

   The button changes to:

   

2. Click on an atom in a residue in the Workspace to select all the atoms in that residue.

2.6.2 Picking Tools

The picking tools are embedded in each panel in which you need to select atoms to apply an operation. The picking tools in a panel can include one or more of the following:

- Pick option menu—Allows you to choose an object type. Depending on the operation to be performed, you can choose Atoms, Bonds, Residues, Chains, Molecules, or Entries, then click on an atom in the Workspace to perform the action on all the atoms in that structural unit.

  The Pick option menu varies from panel to panel, because not all object types are appropriate for a given operation. For example, some panels have only Atoms and Bonds in the Pick option menu.

- All button—Performs the action on all atoms in the Workspace.
- Selection button—Performs the action on any atoms already selected in the Workspace.
- Previous button—Performs the action on the most recent atom selection defined in the Atom Selection dialog box.
- Select button—Opens the Atom Selection dialog box.
- ASL text box—Allows you to type in an ASL expression for selecting atoms.

  ASL stands for Atom Specification Language, and is described in detail in the *Maestro Command Reference Manual*.

- Clear button—Clears the current selection

  

- Show markers option—Marks the selected atoms in the Workspace.

For example, to label atoms with the Label Atoms panel:

1. Choose Atom Labels from the Display menu.
2. In the Composition folder, select Element and Atom Number.
3. In the picking tools section at the top of the panel, you could do one of the following:
   - Click Selection to apply labels to the atoms already selected in the Workspace (from the previous example).
   - Choose Residues from the Pick option menu and click on an atom in a different residue to label all the atoms in that residue.

2.6.3 The Atom Selection Dialog Box

If you wish to select atoms based on more complex criteria, you can use the Atom Selection dialog box. To open this dialog box, choose Select from a button menu or click the Select button in a panel. See Section 5.3 of the *Maestro User Manual* for detailed instructions on how to use the Atom Selection dialog box.

2.7 Scripting in Maestro

Although you can perform nearly all Maestro-supported operations through menus and panels, you can also perform operations using Maestro commands, or compilations of these commands, called *scripts*. Scripts can be used to automate lengthy procedures or repetitive tasks and can be created in several ways. These are summarized below.

2.7.1 Python Scripts

Python is a full-featured scripting language that has been embedded in Maestro to extend its scripting facilities. The Python capabilities within Maestro include access to Maestro functionality for dealing with chemical structures, projects, and Maestro files.

The two main Python commands used in Maestro are:

- `pythonrun`—executes a Python module. (You can also use the alias `pyrun`.) The syntax is:

  `pythonrun` *module*`.`*function*

- `pythonimport`—rereads a Python file so that the next time you use the `pythonrun` command, it uses the updated version of the module. (You can also use the alias `pyimp`.)

From the Maestro Scripts menu you can install, manage, and run Python scripts. For more information on the Scripts menu, see Section 13.1 of the *Maestro User Manual*.

For more information on using Python with Maestro, see *Maestro Scripting with Python*.

2.7.2 Command Scripts

All Maestro commands are logged and displayed in the Command Script Editor panel. This means you can create a command script by performing the operations with the GUI controls, copying the logged commands from the Command History list into the Script text area of the panel, then saving the list of copied commands as a script.

To run an existing command script:

1. Open the Command Script Editor panel from the Edit menu in the main window.
2. Click Open Local and navigate to the directory containing the desired script.
3. Select a script in the Files list and click Open.

   The script is loaded into the Script window of the Command Script Editor panel.
4. Click Run Script.

Command scripts cannot be used for Prime operations.

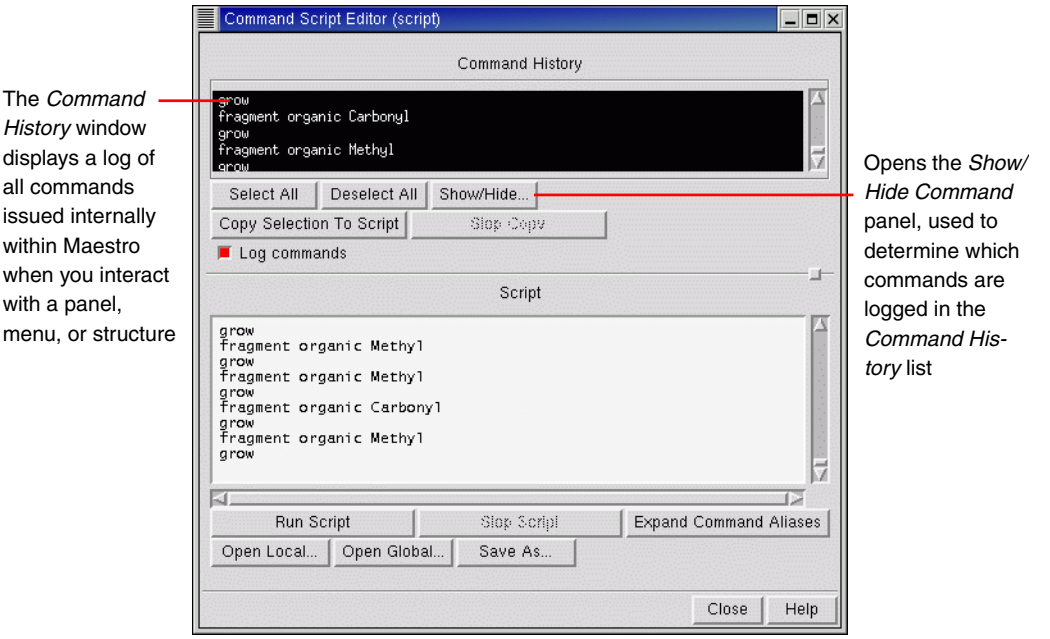

Figure 2.5. The Command Script Editor ***panel.***

2.7.3 Macros

There are two kinds of macros you can create: named macros and macros assigned to function keys F1 through F12.

To create and run a named macro:

1. Open the Macros panel from the Edit menu in the main window.
2. Click New, enter a name for the macro, and click OK.
3. In the Definition text box, type the commands for the macro.
4. Click Update to update the macro definition.
5. To run the macro, enter the following in the command input area in the main window:

   `macrorun` *macro-name*

   If the command input area is not visible, choose Command Input Area from the Display menu.

To create and run a function key macro:

1. Open the Function Key Macros panel from the Edit menu in the main window.
2. From the Macro Key option, select a function key (F1 through F12) to which to assign the macro.
3. In the text box, type the commands for the macro.
4. Click Run to test the macro or click Save to save it.
5. To run the macro from the main window, press the assigned function key.

For more information on macros, see Section 13.5 of the *Maestro User Manual*.

2.8 Specifying a Maestro Working Directory

When you use Maestro to launch Prime jobs, Maestro writes job output to the directory specified in the Directory folder of the Preferences panel. By default, this directory (the file I/O directory) is the directory from which you started Maestro.

To change the Maestro working directory:

1. Open the Preferences panel from the Maestro menu.
2. Click the Directory tab.
3. Select the directory you want to use for reading and writing files.

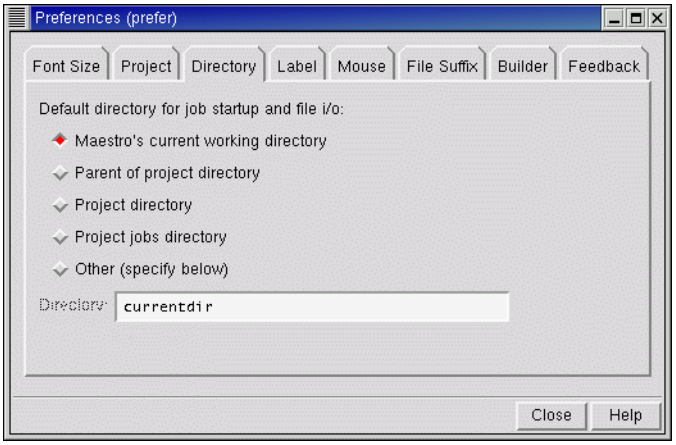

Figure 2.6. T

You can also set other preferences in the Preferences panel. See Section 12.2 of the *Maestro User Manual* for details.

2.9 Undoing an Operation

To undo a single operation, click the Undo button in the toolbar, choose Undo from the Edit menu, or press CTRL+Z. The word Undo in the menu is followed by text that describes the operation to undo. Not all operations can be undone: for example, global rotations and translations are not undoable operations. For such operations you can use the Save view and Restore view buttons in the toolbar, which save and restore a molecular orientation.

2.10 Running and Monitoring Jobs

Maestro has panels for each product for preparing and submitting jobs. To use these panels, choose the appropriate product and task from the Applications menu and its submenus. Set the appropriate options in the panel, then click Start to open the Start dialog box and set options for running the job. For a complete description of the Start dialog box associated with your computational program, see your product's User Manual. When you have finished setting the options, click Start to launch the job and open the Monitor panel.

The Monitor panel is the control panel for monitoring the progress of jobs and for pausing, resuming, or killing jobs. All jobs that belong to your user ID can be displayed in the Monitor panel, whether or not they were started from Maestro. Subjobs are indented under their parent in the job list. The text pane shows various output information from the monitored job, such as the contents of the log file. The Monitor panel opens automatically when you start a job. If it is

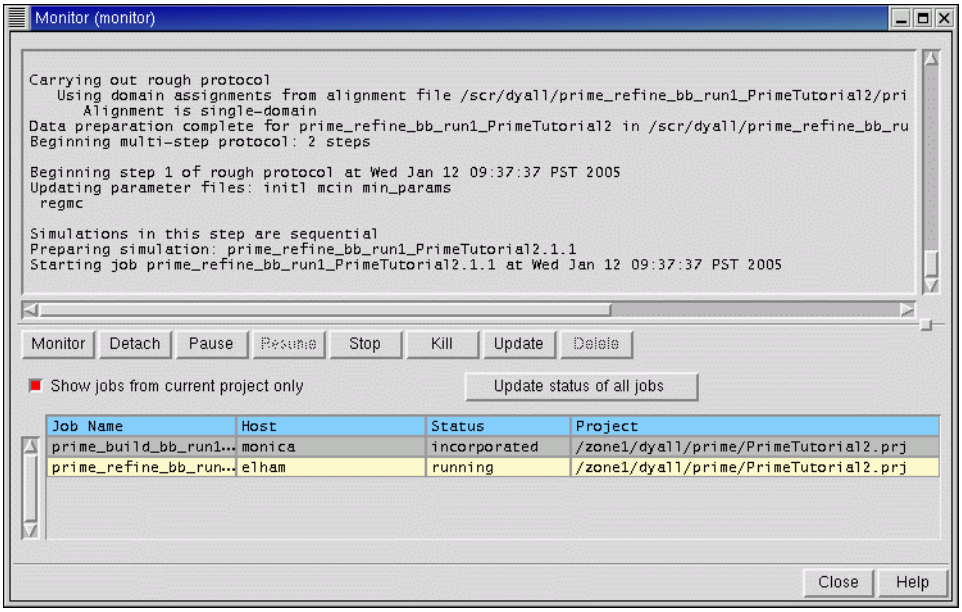

Figure 2.7. The** Monitor **panel.

not open, you can open it by choosing Monitor from the Applications menu in the Maestro main window.

While jobs are running, the Detach, Pause, Resume, Stop, Kill, and Update buttons are active. When there are no jobs currently running, only the Monitor and Delete buttons are active. These buttons act on the selected job. By default, only jobs started from the current project are shown. To show other jobs, deselect Show jobs from current project only.

When a monitored job ends, the results are incorporated into the project according to the settings used to launch the job. If a job that is not currently being monitored ends, you can select it in the Monitor panel and click Monitor to incorporate the results. Monitored jobs are incorporated only if they are part of the current project. You can monitor jobs that are not part of the current project, but their results are not incorporated. To add their results to a project, you must open the project and import the results.

Further information on job control, including configuring your site, monitoring jobs, running jobs, and job incorporation, can be found in the *Job Control Guide* and the *Installation Guide*.

2.11 Getting Help

Maestro comes with automatic, context-sensitive help (Auto-Help), Balloon Help (tooltips), an online help facility, and a user manual. To get help, follow the steps below:

- Check the Auto-Help text box at the bottom of the main window. If help is available for the task you are performing, it is automatically displayed there. It describes what actions are needed to perform the task.
- If your question concerns a GUI element, such as a button or option, there may be Balloon Help for the item. Pause the cursor over the element. If the Balloon Help does not appear, check that Show Balloon Help is selected in the Help menu of the main window. If there is Balloon Help for the element, it appears within a few seconds.
- If you do not find the help you need using either of the steps above, click the Help button in the lower right corner of the appropriate panel. The Help panel is displayed with a relevant help topic.
- For help with a concept or action not associated with a panel, open the Help panel from the Help menu or press CTRL+H.

If you do not find the information you need in the Maestro help system, check the following sources:

- The *Maestro User Manual*
- The Frequently Asked Questions page, found at http://www.schrodinger.com/Support/faq.html

You can also contact Schrödinger by e-mail or phone for help:

- E-mail: help@schrodinger.com
- Phone: (503) 299-1150

2.12 Ending a Maestro Session

To end a Maestro session, choose Quit from the Maestro menu. To save a log file with a record of all operations performed in the current session, click Quit, save log file in the Quit panel. This information can be useful to Schrödinger support staff when responding to any problem you report.

Comparative Modeling Tutorial

Below is a step-by-step tutorial that takes you through the Comparative Modeling path of Prime–Structure Prediction and demonstrates the use of stand-alone Prime–Refinement. You will be building and refining a model of a query sequence for which a sequence homolog can be identified using BLAST. While the tutorial is self-contained, you may find it useful to refer to the *Prime User Manual* or the online help (click the Help button in any Prime panel) for more detailed information about the individual programs that make up the Comparative Modeling path.

3.1 Importing the Query Sequence

The query sequence that will be used is closely related to that of phosphoglycerate kinase from *Pyrococcus furiosus*, but has been modified slightly to provide a case that best demonstrates various features of Prime's Comparative Modeling path:

```
>Query
YNRTVFLRVDLNSPMSNGKVQSDARFRAVLPTIKYLIESGAKVVVGTHQGKEYSTTEEHARILSELLNMH
VEYVEDYAIFGISKARERAAMKPGEVIVLENLRFSAEEFVRKLSQVIDLVVNDAFAAAHRSQPSLVGFAR
IKPMIMGFL
```

In this section, you will copy the query sequence from the tutorial directory and import it into Prime as the first step of the Structure Prediction workflow.

1. Copy the sequence file for this tutorial into your working directory:

   `cp $SCHRODINGER/psp-v`*version*`/tutorial/PrimeTutorial1.fasta` *dir*

2. Start Maestro by entering the command:

   `$SCHRODINGER/maestro &`

3. On the main toolbar, click the Save as button:

   

   The Save As Project dialog box is displayed.

4. Type `PrimeTutorial1` in the Project text box, and click Save.

   You are now working in a named project (not a scratch project) called PrimeTutorial1.prj.

5. Choose Structure Prediction from the Prime submenu of the Applications menu.

   The Structure Prediction panel opens at the first step, Input Sequence.

6. Click From File and select `PrimeTutorial1.fasta`, then click Read.

   The sequence is displayed in the Prime sequence viewer (Figure 3.1). At this stage, there is no structure to display in the Workspace.

   Unlike the Prime sequence viewer, the Workspace sequence viewer in the lower part of the Maestro main panel displays sequences only for named entries in a project. Until the

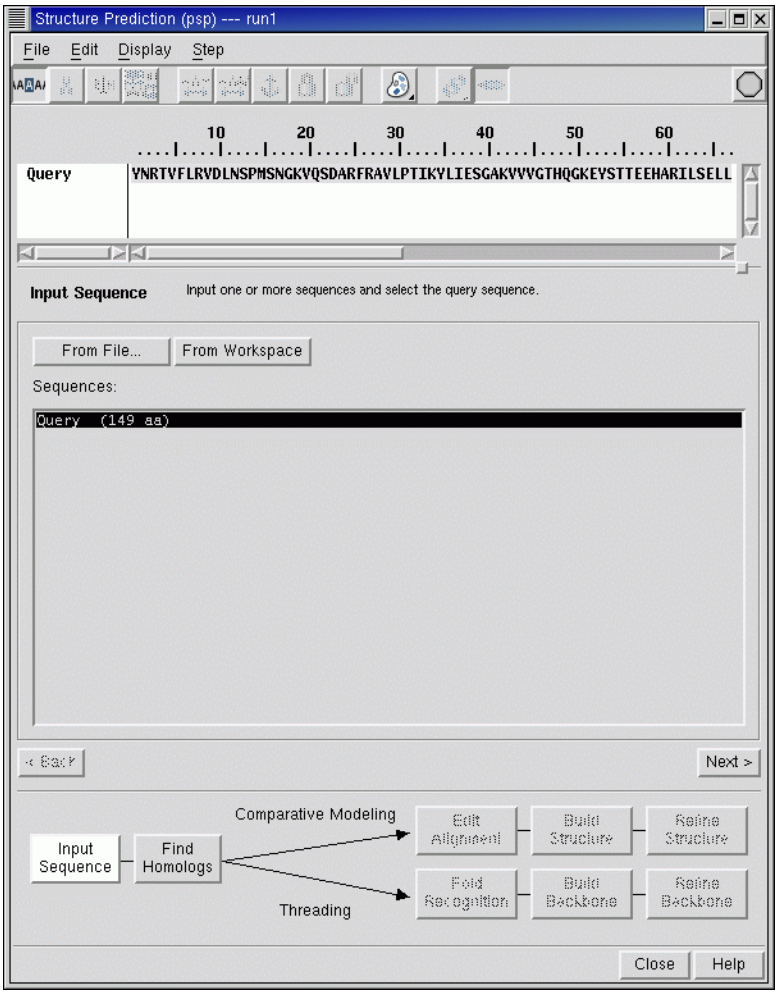

Figure 3.1. The Input Sequence ***step after import.***

end of this tutorial, when the finished structure is added to the Project Table, the Workspace sequence viewer remains empty.

7. Click Next to proceed to the next step, Find Homologs.

3.2 Finding Sequence Homologs

In this step, you will search for homologous proteins with known structure using BLAST, then select one homolog as a template.

1. Click Search.

   The Find Homologs Run Search job is started.

This search usually takes less than 1 minute on a 1-GHz processor. When the job finishes, a list of potential templates is displayed in the Homologs table. The highest-scoring template is selected by default, as shown in Figure 3.2.

The PDB and BLAST databases provided are continually being updated. Therefore, the rank order and scores of the homologs found might differ slightly from that shown.

2. Select the 1VPE template by clicking its row. This template is the first- or second-ranked template in the Homologs table.

   The BLAST alignment between the template and query sequences is displayed in the Prime sequence viewer, along with the secondary structure assignment of the template. In addition, the selected template is displayed in the Workspace.

3. Zoom in on the region of the template that is aligned to the query (the colored region of the ribbon representation.) and manipulate the view to resemble Figure 3.3.

4. Once you are satisfied with the view, save the view so you can easily return to it. To do this, click the Save View button on the Maestro toolbar:

(Optional) To obtain HMMER/Pfam family and sequence data:

1. Click Find Family.

   This job should take 2 to 3 minutes to complete. A Hidden Markov Model (HMM) is generated from a multiple sequence alignment and used to identify the query family and provide information about which residues are conserved in the consensus sequence.

   When the job finishes, the family appears in the Query family name text box.

2. In the Prime sequence viewer, click the plus sign (+) to the left of the sequence name.

   The Pfam sequence data is displayed.

3. Choose Legend from the Display menu or from the right-click menu in the sequence viewer.

   The Legend panel is displayed.

4. Click the Symbols tab and select `Pfam/HMMER` from the Sequence data menu.

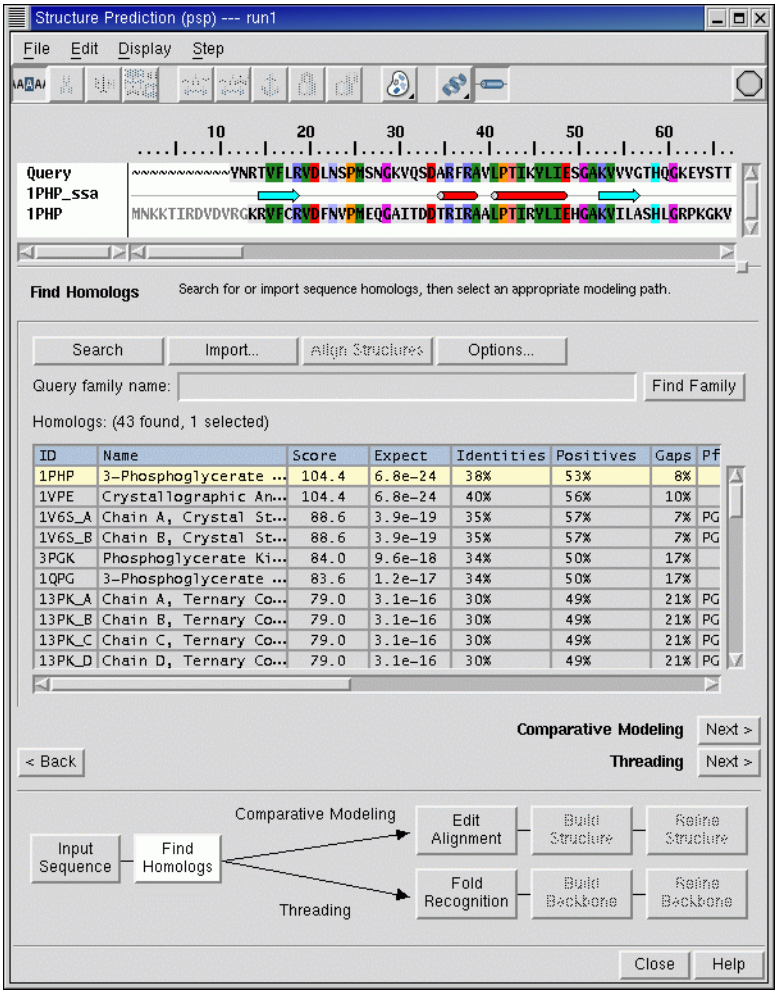

Figure 3.2. The Find Homologs ***step after searching for homologs.***

The key for the HMM consensus sequence is displayed in the sequence viewer. The explanation of the symbols is as follows:

Capital letters	Highly conserved positions
Lowercase	Matches HMM
+	Match is conservative according to HMM
Blank	Does not match HMM

5. Click Close.

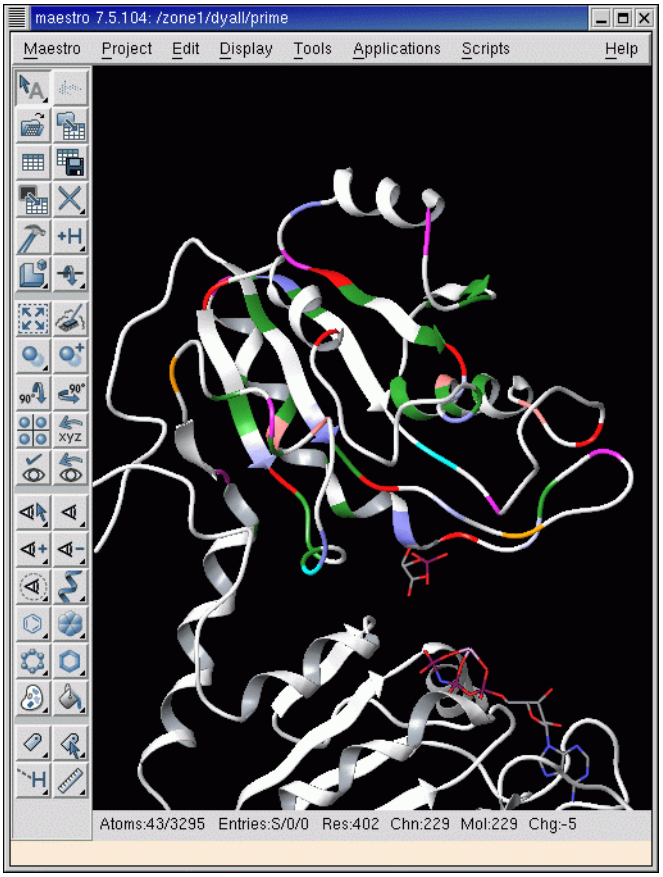

Figure 3.3. The `1VPE` template, showing the region aligned with the query.

To continue to the next step in the Comparative Modeling path:

1. Ensure that 1VPE is still selected.
2. Click the Next button to the right of the words Comparative Modeling.

   The next step is Edit Alignment. The template is again automatically fit to fill the Workspace.
3. To return the view to the one you saved in the previous step, click the Restore view button on the Maestro toolbar.

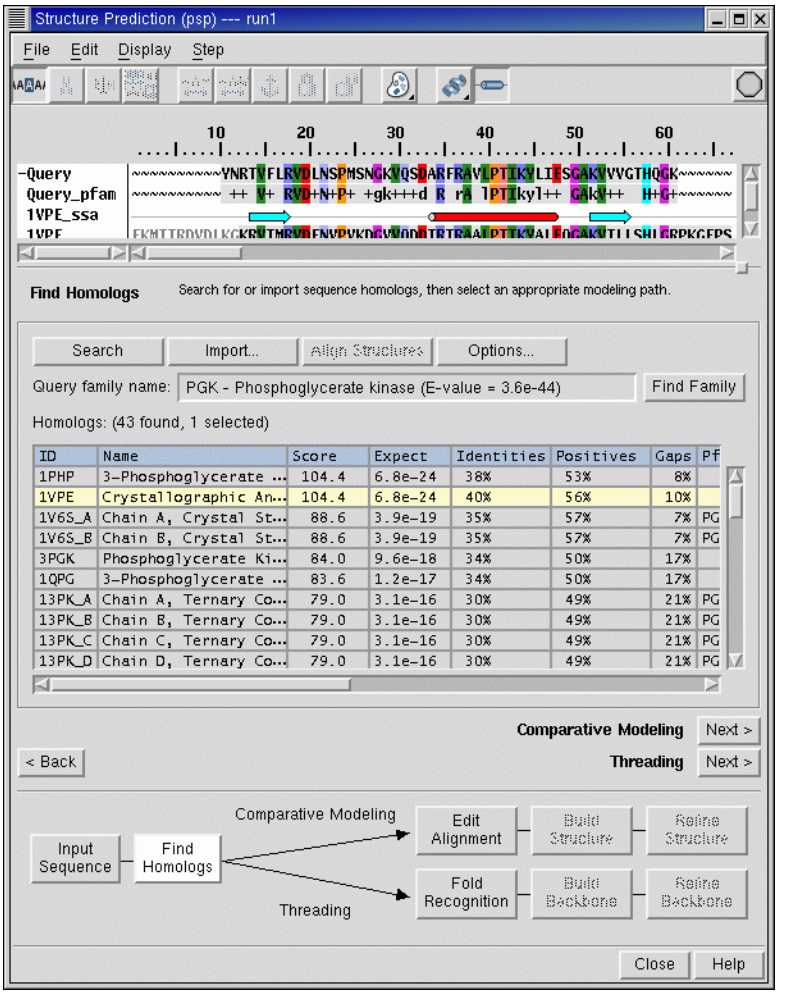

Figure 3.4. The Find Homologs ***step after Find Family and selection of 1VPE template.***

3.3 Editing the Alignment

Because the alignment provided by the Find Homologs step is based only on sequence information, there is room for improvement. For example, the default alignment has placed a gap at query residue His59, which corresponds to the middle of a helix in the template (Figure 3.5). Therefore, it is unlikely that the alignment returned by BLAST is correct in this region. This can be rectified either by hand-editing the alignment or by using the Prime Align program, which takes secondary structure into account.

Before making changes to the BLAST alignment, save the current run:

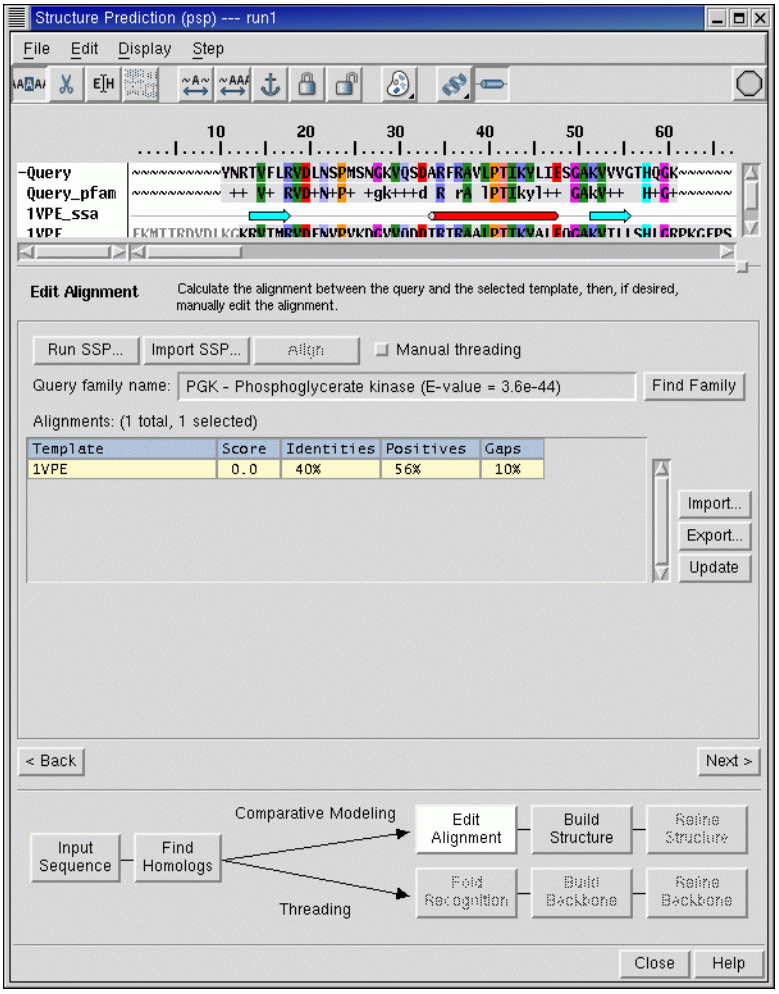

Figure 3.5. Initial view of the Edit Alignment ***step.***

1. From the File menu, choose Rename.
2. Enter `Blast_Alignment` in the dialog box, then click OK.

Name the new run you will be working in:

3. From the File menu, choose Save As.
4. Type `New_Alignment` in the text box and click OK.

   The `New_Alignment` run is the one that is now open. The `Blast_Alignment` run has been closed, but can be reopened at any time.

In order to deal with the fact that secondary structure prediction is only about 75% accurate (for example, visit the EVA site at http://cubic.bioc.columbia.edu/eva/sec/res_sec.html for more details), Prime supports running two distinct secondary structure prediction programs. One of these, SSpro, is bundled with Prime. However, the other, PSIPRED, is not. If you have not already done so, you can find out how to obtain third-party programs[1] from the Third Party Programs page of our website.

Now generate secondary structure predictions for the query to help guide the Align program:

5. Click Run SSP to run all available SSP programs.

   If the optional SSP program PSIPRED was installed (strongly recommended), this job should take about 15 minutes.

   Once the SSP job is completed (when the green octagon turns gray and stops spinning), the secondary structure predictions of the query are displayed in the sequence viewer, as in Figure 3.6.

This and subsequent operations may produce different views of the structure in the Workspace. Click Restore view as needed.

6. Click Align.

   The Prime Align program starts running. This job should take about 10 minutes.

   Once the Align job is completed, the new alignment is displayed in the sequence viewer and the values in the Alignments table are updated. The template's Score, which was 0.0 prior to running the Align program, is now a non-zero number. In addition to some other minor changes in the alignment, the Align program has moved the gap at His59 to an adjacent loop. This makes more physical sense and is likely to result in a more accurate homology model.

1. Please see the notice regarding third party programs and third party web sites on the copyright page at the front of this document.

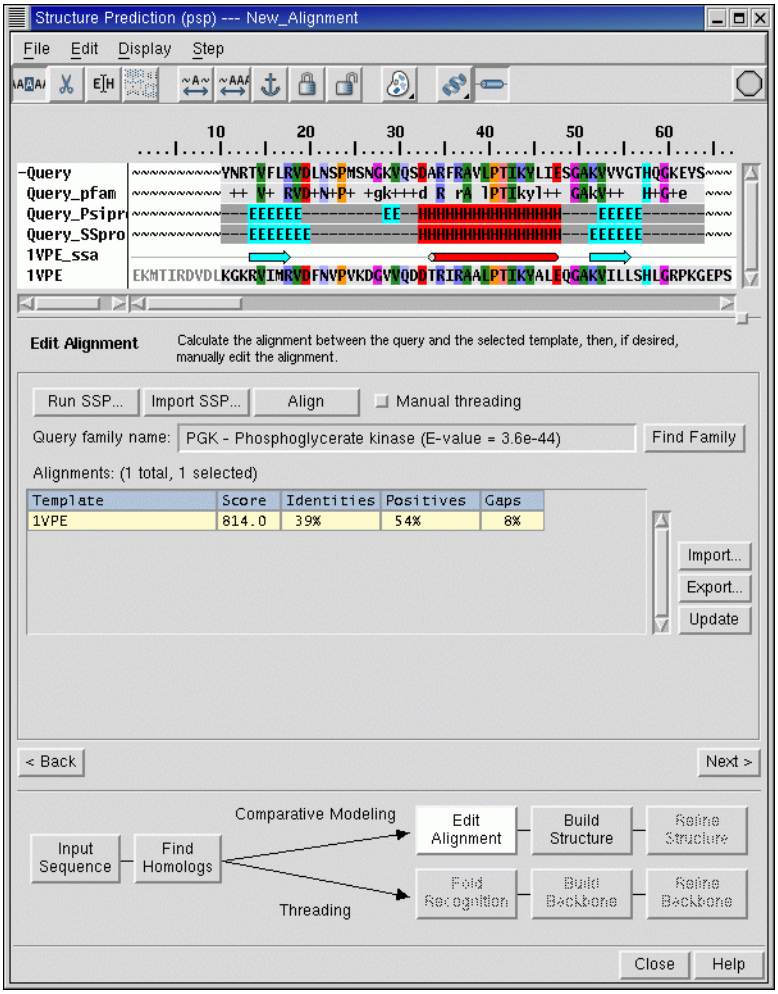

Figure 3.6. The Edit Alignment ***step after running SSP and Align.***

7. (Optional) The true secondary structure of the template is shown graphically in the sequence viewer above the template sequence. To get an indication of the accuracy of the SSP programs in particular regions of the sequence, run the SSP programs on the template: right-click on the template in the sequence viewer and choose Run SSP from the menu.

While the Align program took secondary structure into account in producing the alignment between query and template, it did not explicitly consider tertiary structure. You will next perform some manual editing of the alignment that accounts for tertiary structure.

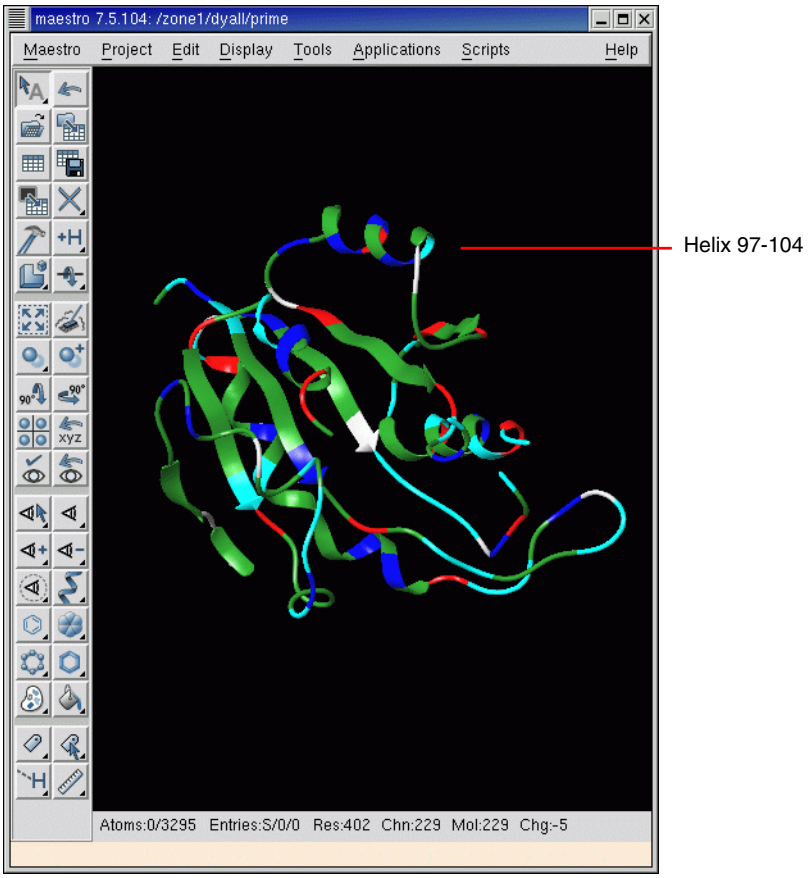

Figure 3.7. The template colored by the query's residue property.

8. Select Manual Threading to enter the Manual Threading mode.

   Aligned residues in the template in the Workspace are now colored according to the query's Residue Property (Figure 3.7). That is, they are colored according to the residue type to which they will be converted once the model is built. Residues that are not being used in the current alignment are undisplayed, revealing where gaps exist in the alignment.

9. Examine the structure to confirm that hydrophobic residues (green) are directed toward the interior of the protein and charged residues (negative: red, positive: blue) are directed toward solvent (polar uncharged residues are colored cyan).

   The only exception is Helix 97-104 (template numbering), shown in Figure 3.7. To find this helix in the Workspace:

a. Scroll the Prime sequence viewer so that the ruler position 100 is in the center of the sequence viewer.

b. Select the residues in the template of the helix that starts at ruler position 98 (Asp97 in the template).

The selected residues are highlighted in the Workspace with yellow markers.

10. To remove the markers, click in a blank area in the sequence viewer.

    Several charged residues appear to be directed towards the interior of the protein, which is likely to result in buried charges in the model once built. This problem can be rectified by manually editing the alignment in this region. Fortunately, there is a two-residue gap near the helix that allows for some flexibility in the local alignment.

11. Change to Slide Freely mode by clicking Slide Freely on the Prime toolbar:

    

12. Drag residue Leu106 (of the template) to the left by two positions.

    The original gap is closed, and a new C-terminal gap is created.

13. Click Update to view the effect of the change in the Workspace.

    The problematic charged residues are now mapped to residues directed outward, which is more physically reasonable (see Figure 3.8).

14. Now that an optimal alignment between query and template has been generated, click Next to proceed to the Build Structure step.

3.4 Building a Model Structure

The Build Structure program builds insertions, closes gaps, and predicts side-chain conformations of non-conserved residues to produce a model with no unphysical clashes. However, it does this efficiently, without extensive conformational sampling. The structure produced in the Build Structure step is likely to represent only a local energy minimum and not the global minimum. Therefore, regions with gaps in the alignment are likely to require refinement in the Refine Structure step.

In this part of the exercise, you will construct a homology model based on the alignment produced in the previous step and that includes the template ligand 3PG (3-phosphoglyceric acid).

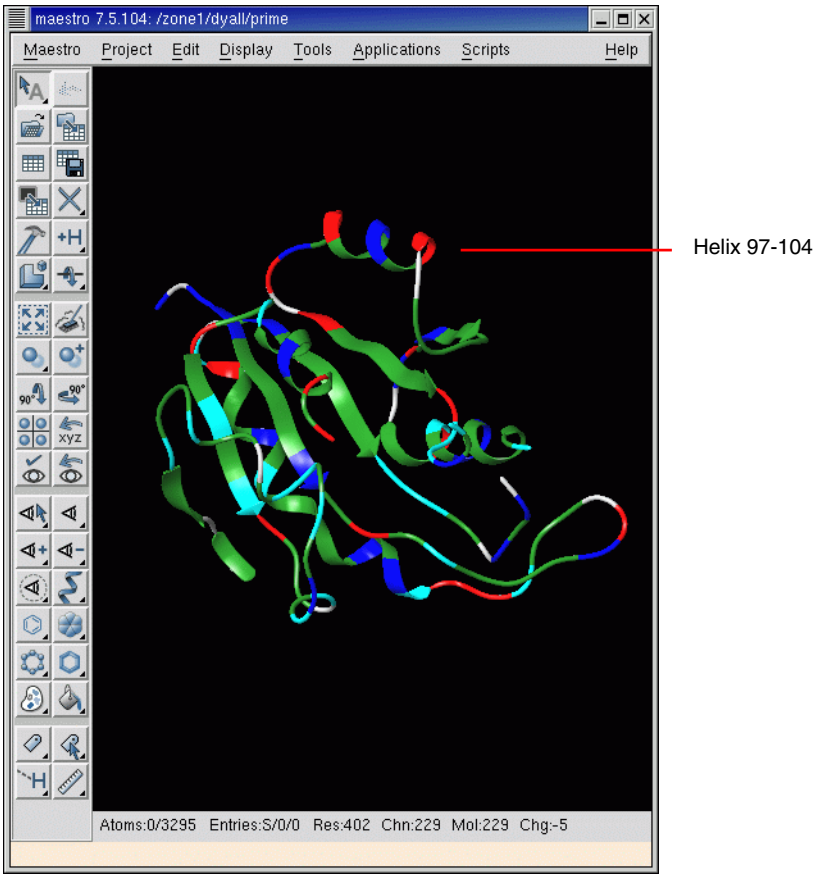

Figure 3.8. Helix from Figure 3.7 after hand editing in Manual Threading ***mode.***

1. Select the ligand 3PG from the Include ligand and cofactors list .

   The selected ligand is highlighted in the Workspace.

2. Click Build.

   This job takes about 15 minutes on a 1-GHz processor.

Once the model-building calculation is complete, the model is displayed in the Workspace superimposed on the template (Figure 3.10).

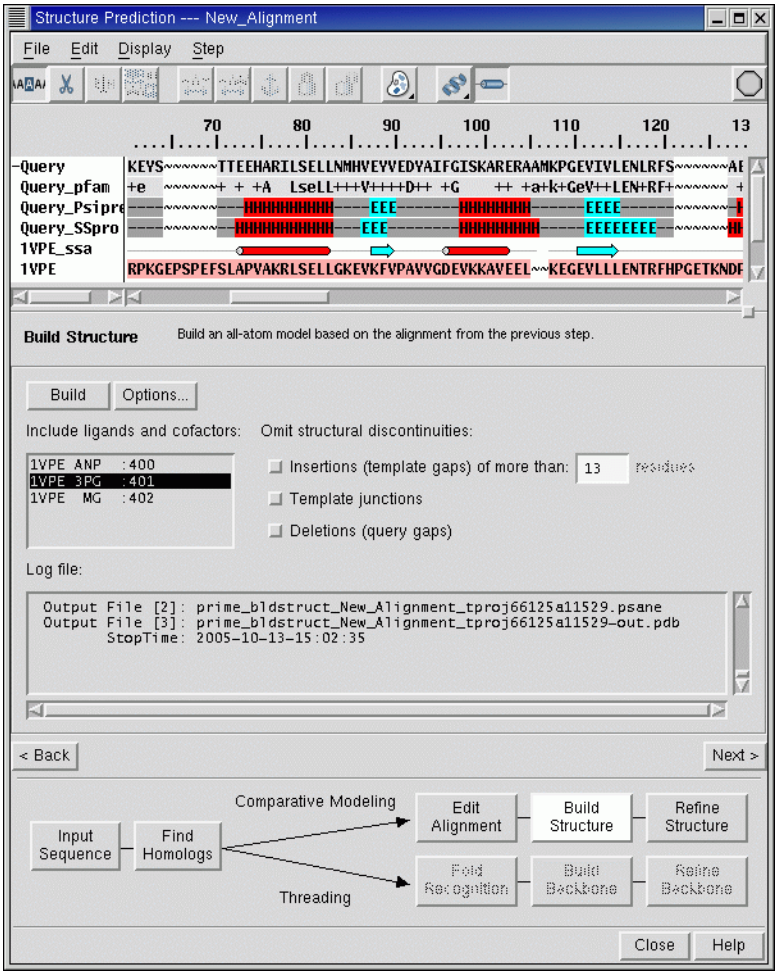

Figure 3.9. The Build Structure ***step after running Build.***

Residues 65-70 (ruler position) of the template needed to be removed from the model. To see these residues highlighted in the Workspace:

3. Click the Selection button on the Prime toolbar:

4. Select the residues in the Prime sequence viewer. (Figure 3.9).

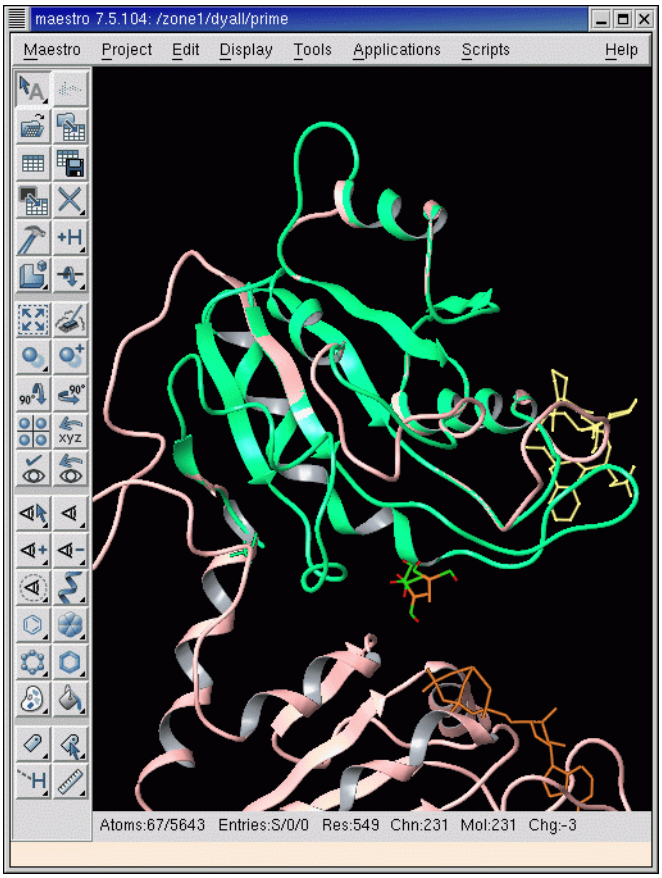

Figure 3.10. Residues 65 through 70 (yellow) selected in the sequence viewer.

Figure 3.9 also shows that a gap between query residue Ser54 (ruler position 64) and Thr55 needed to be closed by Build Structure. As explained above, this region should be targeted for refinement.

5. Click Next to proceed to the Refine Structure step.

3.5 Refining Target Regions of the Structure

3.5.1 Refining Loops

To improve the structure most efficiently, you should focus refinement efforts on areas of the structure that are likely to be problematic. In general terms, this means refining loops (particularly where insertions have been made or gaps closed) and re-predicting side-chain conformations. A particular structure may also have atom position clashes, non-ideal bond lengths and angles, and residues with unfavorable energies.

There are three refinement tasks available in the Refine Structure step: Refine loops, Predict side chains, and Minimize. Refine loops is the default task.

1. In the table of loops, click on the word `loop3`.

   Loop 3 is selected. Yellow markers appear in the Workspace to indicate the location of this loop in the structure.

   Refinement of loops of six or more residues should be performed using extended, not default, sampling. You can change the sampling method in the Refine Structure - Options panel. In this exercise, the length of the loop will be edited so that the faster default sampling can be used.

2. Change the beginning residue (Res1) to 53 for `loop3`.

   You can edit the table by clicking in a cell and entering a new value.

3. Click the check box for `loop3` in the Run column.

4. Click Run.

   The refinement calculation is started. This job takes about 30 minutes. To monitor its progress, click the spinning octagon in the upper right corner. The Monitor panel is displayed and lists the log file.

   When the job finishes, the predicted structure and energy appear in the Structures table, as shown in Figure 3.11.

While we have been referring to the calculation that was just performed as a *refinement*, it is more accurately described as a *prediction*. The so-called refinement of loop 53-56 was in fact an ab initio loop prediction, in that the program initially deleted the loop, reconstructed it in a particular way, and then exhaustively sampled it to identify the lowest energy conformation.

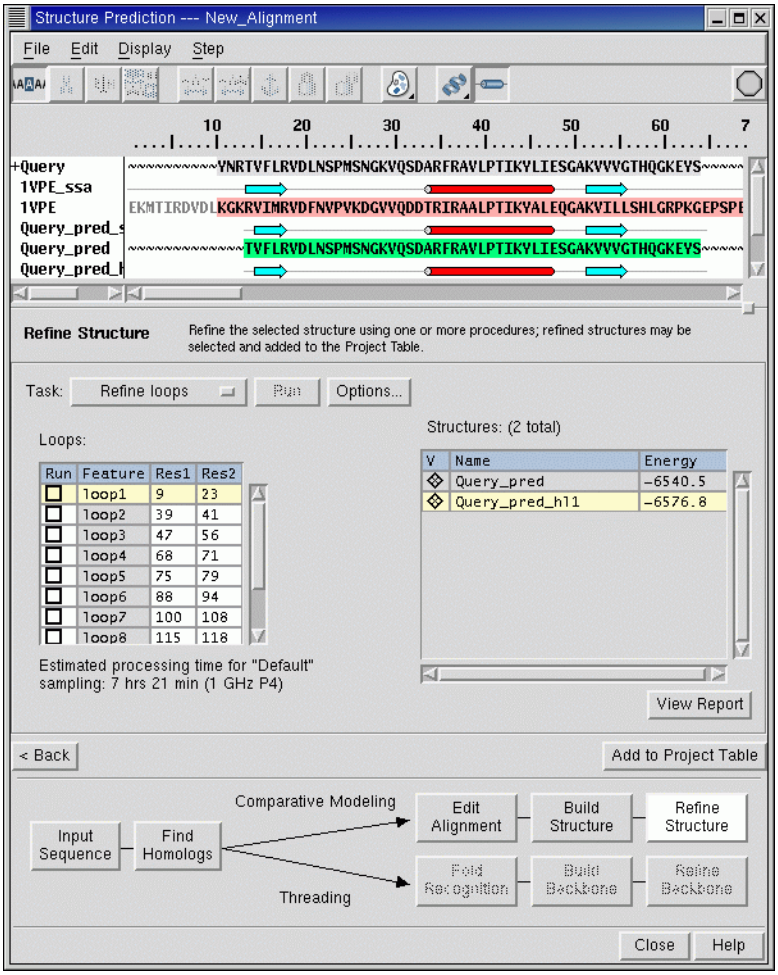

Figure 3.11. The Refine Structure ***step after loop refinement.***

Refinement of loops that are less than 9 residues long yield excellent results in a large majority of cases. Loops 10 to 12 residues long yield very good results in a majority of cases. Loops 13 to 15 residues long produce a low energy conformation most of the time, but probably not the global minimum. Loops 16 to 20 residues long produce a low energy conformation, but refinement of loops this long will take on the order of 1-2 days. Loops longer than 20 residues long should not be attempted, partly because of the sampling problem, but also because the run times will be unreasonably long.

3.5.2 Minimizing Target Regions

Since only side chains (not the backbone) of residues within 7.5 Å were sampled during the previous loop refinement, it is not unreasonable to minimize the local environment of the loop before considering refinement complete. This can be done in two ways:

- Continue to work within the Refine Structure step of Prime–SP
- Use the stand-alone Refinement panel.

To continue working within the Refine Structure step:

1. With structure `Query_pred_hl1` selected in the table, choose Minimize from the Task menu.
2. Click Select.

   The Atom Selection dialog box is displayed.
3. In the Residue Number text box, enter `53-56` and click Add.
4. Click Proximity.

   The Proximity dialog box is displayed.
5. Type `8.5` in the text box, select Residues, and click OK.
6. Click OK in the Atom Selection dialog box.

   Loop 53-56 and all residues within 8.5 Å of this loop are now selected.
7. Click Run.

   The minimization job starts. Once completed, the new structure appears at the bottom of the table, and is displayed in the Workspace (See Figure 3.12 and Figure 3.13). This job should take about 5 minutes.
8. When the prediction job finishes, click Add to Project Table.

   The Project Table panel opens with the selected structure as an entry.
9. Close the Prime–SP panel.
10. Click the In check box in the Project Table panel to include the entry in the Workspace.

    It is now possible to use the refined homology model as input to other Schrödinger programs.

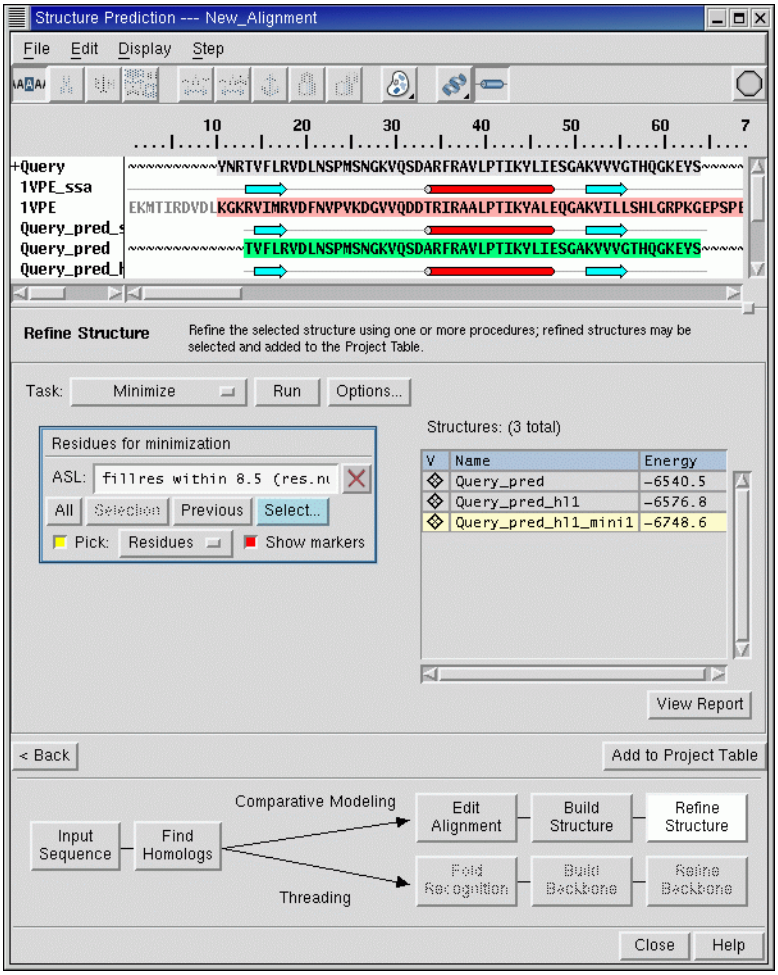

Figure 3.12. The Refine Structure ***step after minimizing residues near the loop prediction.***

Structures visible in the Workspace while working in the Prime–SP interface are scratch entries (not yet part of the Project Table.) The Workspace sequence viewer does not display scratch entries. Now that this structure is a Project Table entry, its sequence and SSA are displayed in the Workspace sequence viewer. The Workspace sequence viewer is not displayed by default. To display it, choose Sequence Viewer from the Display menu.

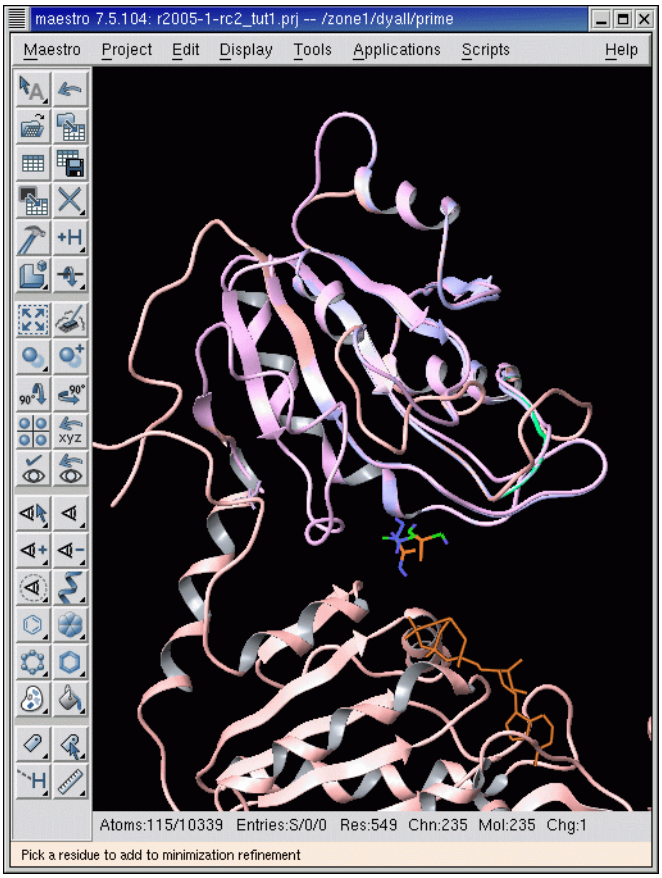

Figure 3.13. The minimized structure.

To use stand-alone Prime–Refinement:

1. Select the predicted structure and click Add to Project Table.

   The Project Table panel for the PrimeTutorial1 project opens with the selected structure as an entry. This step is necessary because the Prime–Refinement module works with structures in Maestro.

2. Click the In check box.

   The entry is included in the Workspace. Structures visible in the Workspace while working in the Prime–SP interface are scratch entries (not yet part of the Project Table.) The Workspace sequence viewer does not display scratch entries. Now that this structure is a Project Table entry, its sequence and SSA are displayed in the Workspace sequence

viewer. The Workspace sequence viewer is not displayed by default. To display it, choose Sequence Viewer from the Display menu.

3. Close the Prime–SP panel.
4. Choose Refinement from the Prime submenu of the Applications menu.

   The stand-alone Prime–Refinement panel is displayed.
5. Choose Minimize from the Task menu.
6. Click Select.

   The Atom Selection dialog box is displayed.
7. In the Residue Number text box, enter `53-56` and click Add.
8. Click Proximity.

   The Proximity dialog box is displayed.
9. Type `8.5` in the text box, select Residues, and click OK.
10. Click OK in the Atom Selection dialog box.

    Loop 53-56 and all residues within 8.5 Å are now selected.
11. Click Start.

    When the job finishes, the minimized structure is automatically incorporated into the project. It is now possible to use the refined homology model as input to other Schrödinger programs.

Threading Tutorial

In practice, the Threading path in Prime–Structure Prediction is used when no sequence homologs can be identified for the query sequence. Because sequence databases are continually expanding, an example chosen for its lack of homologs today may acquire one tomorrow, making it a candidate for the Comparative Modeling path instead. Since the goal of this tutorial is to familiarize you with the steps of the Threading process rather than to solve a research puzzle, the query sequence has been selected and modified for convenience. The Threading steps will be followed regardless of the existence of a sequence homolog.

4.1 Starting the Tutorial

1. In a terminal window, copy the sequence file `PrimeTutorial2.fasta` into your working directory:

   `cp $SCHRODINGER/psp-v`*version*`/tutorial/PrimeTutorial2.fasta` *working_dir*`/ .`

2. Start Maestro by entering the command

   `$SCHRODINGER/maestro &`

3. On the main toolbar, click the Save as button:

   

   The Save As Project dialog box is displayed.

4. Type `PrimeTutorial2` in the Project text box, and click Save.

   You are now working in a named project (not a scratch project) called PrimeTutorial2.

5. Choose Structure Prediction from the Prime submenu of the Applications menu.

   The Prime–Structure Prediction (Prime–SP) panel opens at the first step, Input Sequence.

4.2 Importing the Query Sequence

1. Click From File and select `PrimeTutorial2.fasta`, then click Read.

The sequence is displayed in Prime's sequence viewer. `PrimeTutorial2.fasta` contains the following query:

```
>Query
MKSHKMMGGGISMHYITACLKIISDKDLNEIMKEFKKLEEETNKEEGCITFHAYPLEPSERK
IMLWEIWENEEAVKIHFTKKHTIDVQKQELTEVEWLMKSNVND
```

2. Click Next to proceed to the next step, Find Homologs.

4.3 Searching for Sequence Homologs

In this exercise, you will search for homologous proteins with known structure using BLAST:

1. Click Search.

   The Find Homologs Run Search job is started.

   This search usually takes less than 1 minute on a 1-GHz processor. When the job finishes, a list of potential templates, with the highest-scoring one selected by default, is displayed in the Homologs table.

Regardless of whether a high-homology template is found, in this tutorial you will proceed as though none has been found.

2. Click the Next button to the right of the word Threading to go to Fold Recognition.

4.4 Generating SSPs and Running Fold Recognition

Initially, the Templates table in this step is empty. None of the sequence homologs from the previous step are used in this path. Instead, secondary structure prediction programs are run on the query sequence and the resulting SSPs are used to identify seed templates.

In order to deal with the fact that secondary structure prediction is only about 75% accurate, Prime supports running two distinct secondary structure prediction programs. One of these, SSpro, is bundled with Prime. However, the other, PSIPRED, is not. If you have not already done so, go to the Third Party Programs page of our website to find out how to obtain third-party programs.

1. Click Run SSP.

   If both SSP programs have been installed, both will be run, which may take 5 to 15 minutes.

   Once the SSP job is completed (when the green octagon turns gray and stops spinning), the secondary structure predictions of the query will be displayed in the sequence viewer.

2. Click Search

   The search for templates based on secondary structure predictions starts. This search may take 5 to 10 minutes.

   When the search is complete, the 100 best potential templates are shown in the Templates table, with the top 25 automatically selected (highlighted in yellow).

3. Click Next to proceed to the Build Backbone step.

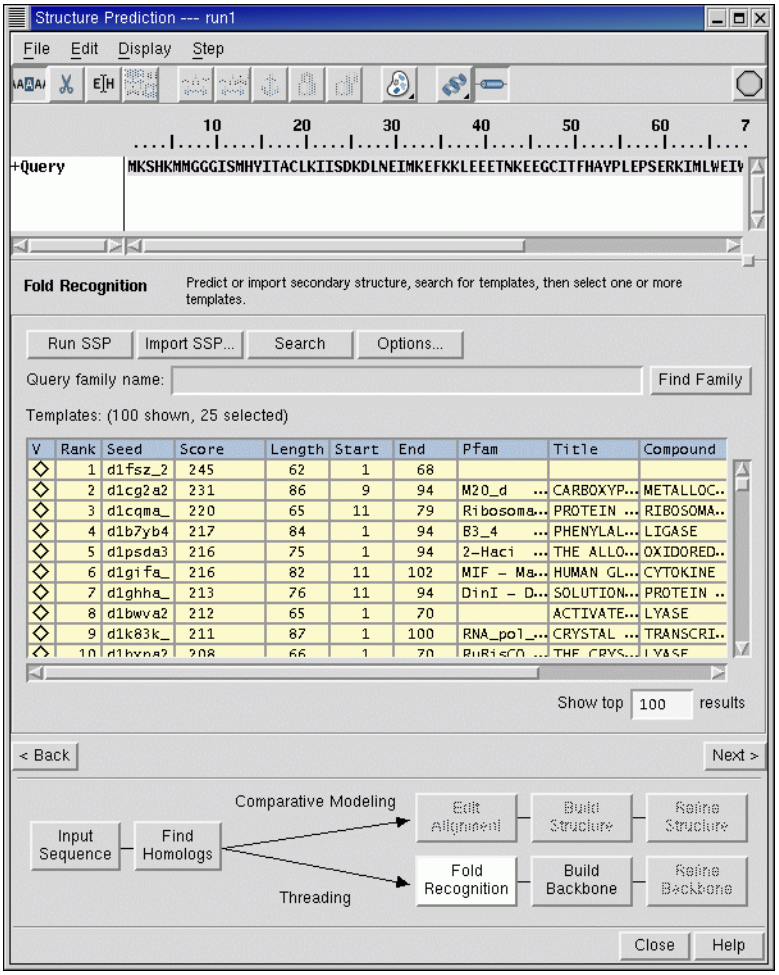

Figure 4.1. The** Fold Recognition **step after search.

4.5 Building Backbone Models

The Structures table is empty when you enter the Build Backbone step. By default, however, up to 20 candidate backbone structures are calculated for each of the 25 seed templates selected in the previous step. This and other settings can be changed by clicking Options and altering the selections in the Build Backbone-Options panel.

The 25 subjobs (one for each template) can be run on multiple processors if they are available. This is also useful when running the next step, Refine Backbone.

1. (Optional) Change the host machine on which the job will be run:
   a. From the File menu, choose Job Options.

      The Structure Prediction - Job Options panel opens, displaying a Jobs table. For each step, the jobs run from that step are listed, with their current Host and Login settings.

   b. Select the Build Backbone step from the Jobs table.
   c. Choose a multiprocessor machine from the Host menu.
   d. Enter the appropriate Login if it differs.
   e. To the right of the words Set for, click Selected.
   f. (Optional), select the Refine Backbone step from the table and repeat Step b through Step e.
2. Click Build.

   The Structure Prediction - Launch Build Backbone dialog box is displayed. It contains information to help you select the number of templates to be run at one time. It also displays current job options and offers an Edit button that opens the Structure Prediction - Job Options panel.

   - Number of selected templates box
   - Run in groups of box
   - Job Settings: Edit button
   - Host box
   - Login box
3. Set the group size to the number of CPUs available on the host machine, or if submitting to a queue, set to the number of selected templates (25).
4. Click OK

   The Build Backbone job starts. Each subjob takes roughly 45 minutes on a single 1-GHz processor.

When the entire Build Backbone job is complete, candidate structures appear in the Structures table, ranked by Preliminary Ranking Score. The top 20 are selected by default. For information on the table columns, see Section 9.3 of the *Prime User Manual*.

By default, none of the structures is shown in the Workspace. Backbone models that have not yet been refined are preliminary and may display nonphysical structures. To view a structure in the Workspace, select the checkbox in the V column.

5. Click Next to go to Refine Backbone.

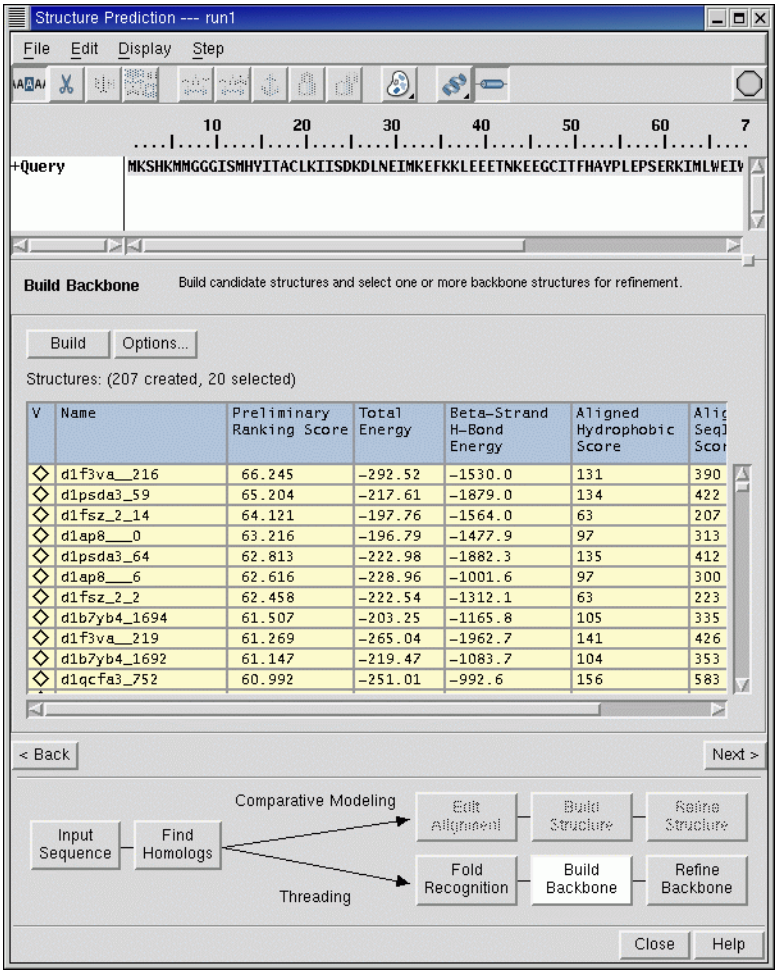

Figure 4.2. The Build Backbone ***step after building.***

4.6 Refine Backbone

The 20 backbone structures chosen for refinement appear in the Composite structures table with their Preliminary Ranking Score.

- Click Refine.

  The backbone refinement job starts.

Note: The subjobs of this job can stall. To ensure that they make progress, open the Monitor panel, select the subjob and click Monitor.

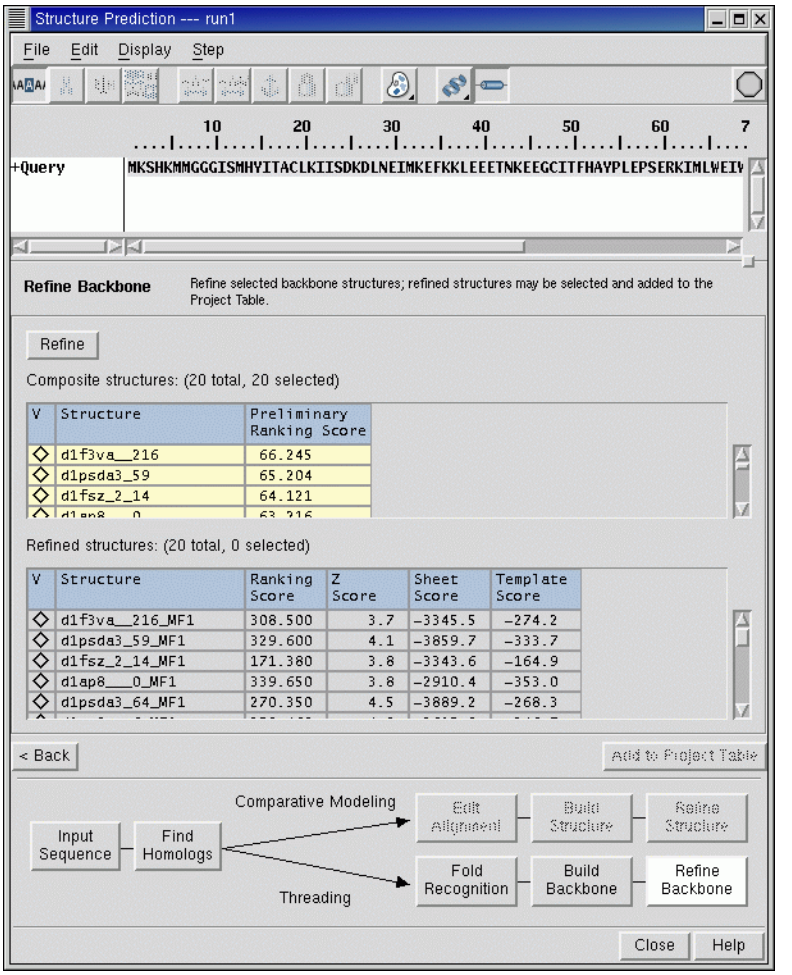

Figure 4.3. The Refine Backbone ***step after refinement.***

When backbone refinement of a structure is complete, it is added to the Refined structures table. For more information about refined backbone structure properties such as Ranking Score, Z Score, Sheet Score, and Template Score, see Section 10.2 of the *Prime User Manual*.

To incorporate refined backbone structures in the Maestro project:

1. Select the refined structures you want to work with further.
2. Click Add to Project Table.

   The project table is displayed. In the project table, the Refine Backbone Ranking Score is shown as the Prime Threading Score.

Getting Help

Schrödinger software is distributed with documentation in PDF format. If the documentation is not installed in `$SCHRODINGER/docs` on a computer that you have access to, you should install it or ask your system administrator to install it.

For help installing and setting up licenses for Schrödinger software and installing documentation, see the *Installation Guide*. For information on running jobs, see the *Job Control Guide*.

Maestro has automatic, context-sensitive help (Auto-Help and Balloon Help, or tooltips), and an online help system. To get help, follow the steps below.

- Check the Auto-Help text box, which is located at the foot of the main window. If help is available for the task you are performing, it is automatically displayed there. Auto-Help contains a single line of information. For more detailed information, use the online help.
- If you want information about a GUI element, such as a button or option, there may be Balloon Help for the item. Pause the cursor over the element. If the Balloon Help does not appear, check that Show Balloon Help is selected in the Help menu of the main window. If there is Balloon Help for the element, it appears within a few seconds.
- For information about a panel or the folder that is displayed in a panel, click the Help button in the panel. The Help panel is opened and a relevant help topic is displayed.
- For other information in the online help, open the Help panel and locate the topic by searching or by category. You can open the Help panel by choosing Help from the Help menu on the main menu bar or by pressing CTRL+H.

  To view a list of all available Prime–related help topics, choose Prime from the Categories menu of the Categories tab. Double-click a topic title to view the topic.

If you do not find the information you need in the Maestro help system, check the following sources:

- *Maestro User Manual*, for detailed information on using Maestro
- *Maestro Tutorial*, for a tutorial on the basic features of Maestro
- *Maestro Command Reference Manual*, for information on Maestro commands
- *Prime User Manual*, for detailed information on using Prime
- Frequently Asked Questions pages, at https://www.schrodinger.com/Prime_FAQ.html

The manuals are also available in PDF format from the Schrödinger Support Center. Information on additions and corrections to the manuals is available from this web page.

If you have questions that are not answered from any of the above sources, contact Schrödinger using the information below.

E-mail: help@schrodinger.com
USPS: 101 SW Main Street, Suite 1300, Portland, OR 97204
Phone: (503) 299-1150
Fax: (503) 299-4532
WWW: http://www.schrodinger.com
FTP: ftp://ftp.schrodinger.com

Generally, e-mail correspondence is best because you can send machine output, if necessary. When sending e-mail messages, please include the following information, most of which can be obtained by entering `$SCHRODINGER/machid` at a command prompt:

- All relevant user input and machine output
- Prime purchaser (company, research institution, or individual)
- Primary Prime user
- Computer platform type
- Operating system with version number
- Prime version number
- Maestro version number
- mmshare version number

Glossary

alignment—The optimal matching of residue positions between sequences, typically a query sequence and one or more template sequences.

anchor—A constraint on alignment set at a given residue position. Alignment changes must preserve the query-template pairing at that residue until the anchor is removed.

ASD—Atom Selection dialog box.

ASL—Atom Specification Language.

button menu—The menu available from a toolbar menu button, which you open by holding down the left mouse button.

Comparative Modeling—Protein structure modeling based on a query-template match with a substantial percentage of identical residues (usually 50% or greater sequence identity).

composite template—A type of template used in the Threading Path, produced from the core (invariable) and variable regions of a family of structurally similar proteins.

constraints—Tools to keep regions of a sequence (alignment constraints) or structure (during minimization) in a particular configuration.

deletions—The residues missing from a query sequence that are present in a template sequence.

entry—A structure or set of structures and associated properties. Entries are represented as a row in the project table, and can be used as input for jobs.

Fold Recognition—The use of secondary structure matching and profiles generated from multiple sequence/structure alignments to find templates when sequence methods are unsuccessful.

gaps—The spaces in an alignment resulting from insertions and deletions.

HETATMs—The atoms of residues, including amino acids, that are not one of the standard 20 amino acids. In PDB files, HETATM.

homolog—A sequence/structure related to the query sequence; i.e., a sequence with many of the same residues in the same patterns as the query sequence. Usually these sequences are derived from the same family and may have similar function.

insertions—The extra residues found in a query sequence that are not found in a template sequence.

loop—A region of undefined secondary structure.

Maestro toolbar—The array of icon buttons which provides tools for common Maestro tasks, located by default along the left side of the main window. There are buttons for operations such as moving structures in the Workspace, changing what is displayed, opening a project, or undoing the most recent Maestro operation.

Main menu bar—The menu bar at the top of the main Maestro window below the Auto-Help window. The main menu bar contains menu titles (Maestro, Project, Edit, etc.) that, when clicked, display menus from which selections can be made.

menu button—A toolbar button that has a menu, which you open by holding down the left mouse button. The button has a black triangle in the lower right corner.

Prime toolbar—The row of icon buttons which provides tools for common Prime tasks, located near the top of the Prime-SP panel.

project—A collection of related data, such as structures with their associated properties. In Prime a project comprises one or more *runs* (executions of the Prime workflow). The project may include data that does not appear in the *project table*.

project table—The Maestro panel associated with a project, featuring a table with rows of entries and columns of properties.

query sequence—A sequence of unknown structure or fold.

Ranking Score—The score used to rank composite templates derived from different seed templates. Generated by the Global Scoring Function in the Threading Path.

refinement—An improvement of a model structure through energy-based optimization of selected regions.

run—A single execution of the Prime workflow using a particular set of choices (of templates, of Paths, and of settings). Each run belongs to a *project*. Runs cannot be saved without saving the project to which they belong.

SSA—Secondary structure assignment.

SSP—Secondary structure prediction.

sequence viewer—An area in which protein sequences are displayed. Right-clicking a sequence opens an *option menu*.There are sequence viewers in the Prime–SP panel and in the Maestro main window. The Prime sequence viewer displays query and template sequences,

including family and conservation data in sequence format, *SSA*s, and *SSP*s. The Workspace sequence viewer displays the sequence and (by default) the SSA for the structures included in the Workspace, provided that they are entries in a named Maestro project.

template sequence—A sequence of known structure and fold used as a basis for building a model of the query.

Threading—A structure prediction process in which *Fold Recognition* is used to define templates, then backbone models are built via alignment to composite templates and refined. May be used when query-template sequence identity is low.

Workspace—The open area in the center of the Maestro main window in which structures are displayed.

Z-Score—Measures the compatibility of the query sequence with the model structure, relative to the compatibility of randomly shuffled sequences of the same composition.

Copyright Notices

C and C++ Libraries for Parsing PDB Records

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